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1 **Environmental conditions and male quality traits simultaneously explain variation of**  
2 **multiple colour signals in male lizards**

3

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25 **Abstract**

- 26 1. Male lizards often display multiple pigment-based and structural colour signals which  
27 may reflect various quality traits (e.g. performance, parasitism), with testosterone (T)  
28 often mediating these relationships. Furthermore, environmental conditions can  
29 explain colour signal variation by affecting processes such as signal efficacy,  
30 thermoregulation, and camouflage. The relationships between colour signals, male  
31 quality traits, and environmental factors have often been analysed in isolation, but  
32 simultaneous analyses are rare. Thus, the response of multiple colour signals to  
33 variation in all these factors in an integrative analysis remains to be investigated.
- 34 2. Here, we investigated how multiple colour signals relate to their information content,  
35 examined the role of T as a potential mediator of these relationships, and how  
36 environmental factors explain colour signal variation.
- 37 3. We performed an integrative study to examine the covariation between three colour  
38 signals (melanin-based black, carotenoid-based yellow-orange, and structural UV),  
39 physiological performance, parasitism, T levels, and environmental factors  
40 (microclimate, forest cover) in male common lizards (*Zootoca vivipara*) from 13  
41 populations.
- 42 4. We found that the three colour signals conveyed information on different aspects of  
43 male condition, supporting a multiple message hypothesis. T influenced only  
44 parasitism, suggesting that T does not directly mediate the relationships between  
45 colour signals and their information content. Moreover, colour signals became more  
46 saturated in forested habitats, suggesting an adaptation to degraded light conditions,  
47 and became generally brighter in mesic conditions, in contradiction with the thermal  
48 melanism hypothesis.

49 5. We show that distinct individual quality traits and environmental factors  
50 simultaneously explain variations of multiple colour signals with different production  
51 modes. Our study therefore highlights the complexity of colour signal evolution,  
52 involving various sets of selective pressures acting at the same time, but in different  
53 ways depending on colour production mechanism.

54

## 55 Résumé

56 1. Les lézards mâles arborent souvent plusieurs signaux colorés de nature pigmentaire et  
57 structurale qui reflètent de multiples traits de qualité (e.g. performance, parasitisme),  
58 et la testostérone (T) joue souvent un rôle de médiateur dans ces relations. En outre,  
59 les conditions environnementales peuvent également expliquer les variations des  
60 signaux colorés en influençant des aspects tels que l'efficacité des signaux, la  
61 thermorégulation ou le camouflage. Les relations entre signaux colorés, traits de  
62 qualité individuelle et facteurs environnementaux ont souvent été analysées  
63 séparément, mais rarement de manière simultanée. Ainsi, la réponse de ces multiples  
64 signaux colorés aux variations de tous ces facteurs reste à explorer dans le contexte  
65 d'une étude intégrative.

66 2. Ici, nous explorons la relation entre ces multiples signaux colorés et leur contenu  
67 informatif, nous examinons le rôle de T comme médiateur potentiel de ces relations et  
68 nous recherchons si les conditions environnementales expliquent la variation de ces  
69 signaux colorés.

70 3. Nous avons mené une étude intégrative afin d'examiner la covariation entre trois types  
71 de signaux colorés (noir produit par la mélanine, jaune-orange produit par les  
72 caroténoïdes et UV produit par des éléments structuraux), la performance  
73 physiologique, le parasitisme, les niveaux de T et les conditions environnementales

74 (e.g. microclimat, couverture forestière) chez des mâles du lézard vivipare (*Zootoca*  
75 *vivipara*) provenant de 13 populations.

76 4. Nos résultats indiquent que les trois signaux colorés transmettent des informations sur  
77 différents aspects de la condition des mâles, en accord avec l'hypothèse de « messages  
78 multiples ». T influence uniquement le parasitisme, suggérant que T n'agit pas en tant  
79 que médiateur des relations entre ces signaux colorés et leur contenu informatif. De  
80 plus, les signaux colorés sont plus saturés dans les habitats les plus forestiers, ce qui  
81 suggère une adaptation à des conditions lumineuses dégradées. Enfin, les signaux  
82 colorés sont plus intenses lorsque les conditions sont mésiques, en contradiction avec  
83 l'hypothèse du mélanisme thermal.

84 5. Nous démontrons que différents traits de qualité individuelle et facteurs  
85 environnementaux expliquent de manière simultanée les variations de multiples  
86 signaux colorés impliquant différents modes de production. Notre étude souligne ainsi  
87 la complexité de l'évolution des signaux colorés, qui implique plusieurs types de  
88 pressions de sélection agissant en même temps mais dans des directions différentes  
89 selon le mode de production.

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91

92 Keywords: Animal communication; Coloration; Performance; Parasitism; Reptile;

93 Testosterone; Ultraviolet; *Zootoca vivipara*

94 **Introduction**

95 The display of conspicuous colours is ubiquitous across species and their role in signalling,  
96 thermoregulation, and camouflage has always intrigued ecologists (Cuthill et al., 2017). When  
97 they act as communication signals, colours evolve primarily in response to selection acting on  
98 the relationship between the signal's properties and its information content, and on its  
99 transmission efficacy towards the receiver (Bradbury & Vehrencamp, 2011), although  
100 thermoregulation and camouflage can also constrain colour signal expression (e.g. de Souza et  
101 al., 2016; Lindstedt et al., 2009). In the context of sexual selection, animals often display a  
102 mosaic of different colours that convey information about individual quality (Bradbury &  
103 Vehrencamp, 2011). Colour signals are produced from the deposition of pigments (e.g.  
104 melanin, carotenoids, pterins) in skin cells, from structural light-scattering and light-reflecting  
105 nanoelements, or both (Shawkey & D'Alba, 2017). These modes of production may entail  
106 differences in cost-benefit balances which may ultimately affect their design and function. For  
107 example, while carotenoid-based colours are physiologically costly to produce (reviewed in  
108 Svensson & Wong, 2011), melanin-based colour signals are thought to be associated mainly  
109 to social costs (reviewed in Roulin, 2016). However, the costs associated with pterin-based  
110 and structural colour signals, if any, remain largely unknown (Andrade & Carneiro, 2021;  
111 Kemp et al., 2012), although social costs are potential candidates (e.g. Kawamoto et al.,  
112 2021). If we are to comprehend how colour signals become evolutionarily stable, it is critical  
113 to understand how the relationships between a colour signal, its information content and  
114 environmental factors vary based on the different production modes of multiple colour  
115 signals.

116 Many animals display multiple colour signals that convey information on various  
117 aspects of male quality during male-male competition and female mate choice (Johnstone,  
118 1996, Endler and Mappes 2017). Several hypotheses have been proposed to explain the

119 evolution of multiple colour signals (Hebets and Papaj, 2005; Bro-Jørgensen 2010). The  
120 “multiple message” hypothesis states that each signal component conveys a distinct  
121 information on male quality. In contrast, the “back-up” hypothesis suggest that multiple  
122 signals provide redundant information, thus compensating for signal unreliability (i.e., coding  
123 errors). Other hypotheses explain multiple signals via several receiver-driven mechanisms, for  
124 example having to do with enhancing conspicuousness (i.e., alerting signals, amplifiers),  
125 receiver psychology with multiple signals improving each other’s discriminability, or sensory  
126 overload (reviewed in Bro-Jørgensen 2010). Recent lines of arguments proposed that multiple  
127 signals coexist because they are shaped by dynamics selection regimes, for example related to  
128 changing environments, sexually antagonistic coevolution, or negative frequency-dependent  
129 selection (Bro-Jørgensen 2010). In this context, some signals would be reliable in some  
130 circumstances, but not in others, and multiple colour signals may have evolved as an adaptive  
131 solution to these fluctuating regimes. Moreover, as colour patches often involve three-  
132 dimensional integumentary structures containing multiple pigments and structural elements,  
133 Grether et al. (2004) underlined their potential to act as multicomponent signals, in which  
134 case they should be included within the ‘multiple signals’ framework described above.

135         Lizards are an ideal model to study the evolution of multiple colour signals, as melanin-  
136 based, carotenoid-based and structural colour signals often co-occur on their body and have  
137 been shown to be honest signals of male quality, through correlations with fitness and assays  
138 of resource-holding potential, such as bite force, locomotor capacity, body condition, or  
139 parasite load (e.g., Martín & López, 2009; Pérez i de Lanuza et al., 2014; Plasman et al.,  
140 2015). Androgens such as testosterone (T) can regulate the expression of sexual signals and  
141 mediate their relationships with their information content through different pathways  
142 depending on colour production mechanisms. For instance, T can promote melanin-based  
143 colour signals by stimulating melanophores or via pleiotropic effects (Roulin, 2016). T can

144 also enhance structural colour signals (e.g. UV and blue) by increasing dermal melanisation  
145 under the iridophore layer (e.g. Quinn & Hews, 2003). Moreover, T can promote carotenoid-  
146 based signals through its action on erythrophores (e.g. Kobayashi et al., 2009), by controlling  
147 carotenoids acquisition and allocation (e.g. Peters, 2007), or regulating carotenoid-related  
148 genes (e.g. Khalil et al., 2020). In parallel, T often influences male growth, leading to sexual  
149 size dimorphism (e.g. Cox et al., 2009) and can enhance some physiological traits such as  
150 endurance (e.g. Sinervo et al., 2000) and bite force (e.g. Husak et al., 2007). In addition, the  
151 immunocompetence handicap hypothesis (ICHH) posits that T plays a central role in colour  
152 signal evolution by maintaining signal honesty (Folstad & Karter, 1992). According to the  
153 ICHH, T secretions regulate colour signal expression, yet depresses the immune system, thus  
154 making individuals more vulnerable to parasites. In addition, elevated plasma T levels may  
155 change male behaviour so as to increase exposure risks to parasites (e.g. Olsson et al., 2000).  
156 In fact, the relationship between T, immunity and parasite load can be more complex.  
157 Although T depresses immune functions (reviewed in Foo et al., 2017), its effect on parasite  
158 load seems to be conditional on parasite type (e.g. Fuxjager et al., 2011). Given all this, T is a  
159 good candidate to mediate the relationships between colour signals, their information content  
160 (e.g. condition, performance, parasite load), and their associated costs (e.g. parasitism). In  
161 contrast, the Hamilton-Zuk hypothesis (Hamilton and Zuk, 1982) predicts that bright colours  
162 correlate with low parasite load, thus signalling an individual's good genes, as a consequence  
163 of a genetic association between colour and immunity. To test these hypotheses, we require  
164 studies that include multiple colour signals (e.g. melanin-based, carotenoid-based, structural),  
165 various male quality traits, several parasite types, and T levels.

166       Environmental conditions may also explain variation in colour signals among  
167 individuals and across species. First, environmental conditions can alter visual  
168 communication through variation in light intensity and spectral composition, and in



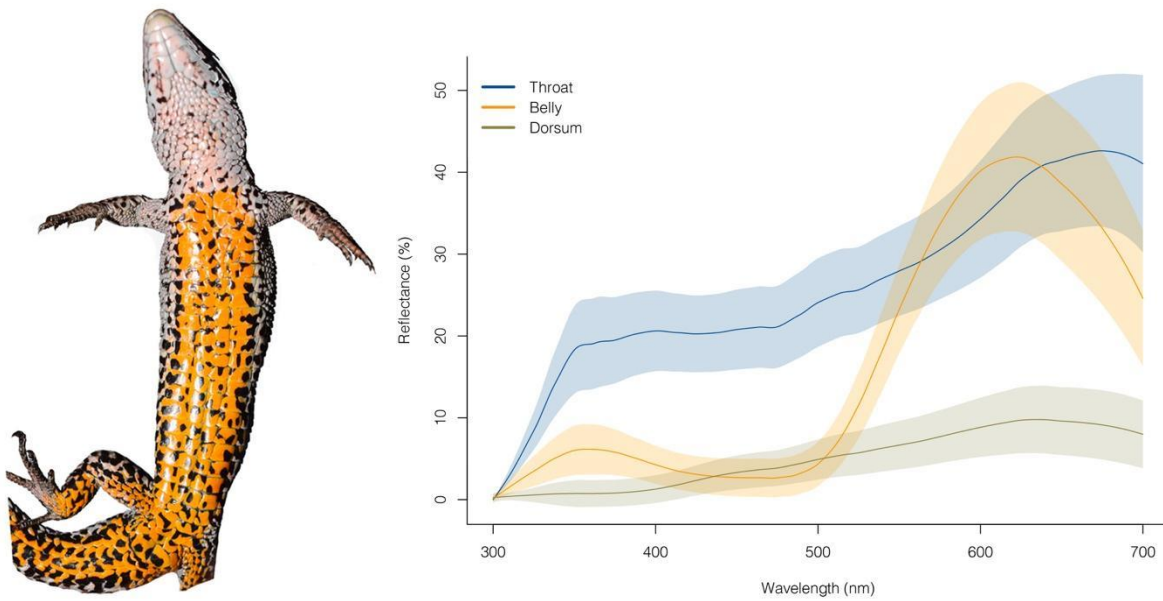
169 background colour (e.g. Fleishman et al., 2020). For example, light conditions and  
170 background colour, which vary along forest cover gradients (Endler, 1993), shape colour  
171 signals in *Anolis* lizards such that signal detectability increases in its local environment  
172 (Fleishman et al., 2020; Leal & Fleishman, 2004). Second, environmental conditions can  
173 influence natural selection on animal colouration, for example in relation to camouflage and  
174 thermoregulation (e.g. Stuart-Fox & Moussalli, 2009). In ectothermic organisms, the thermal  
175 melanism hypothesis predicts that darker individuals heat their body up faster than lighter  
176 ones and should be favoured by natural selection in colder conditions (Clusella Trullas et al.,  
177 2007). Ecogeographical gradients in elevation and latitude, with their concomitant changes in  
178 climate known to constrain thermoregulation processes in ectotherms (e.g. Rozen-Rechels et  
179 al., 2021), have produced colour signal variation consistent with the thermal melanism  
180 hypothesis (Reguera et al., 2014; Svensson & Waller, 2013). Similarly, Gloger's rule, as  
181 revised by Delhey (2019), predicts an association between humid environments and dark  
182 colouration (i.e., melanin deposition) in endotherms, and possibly in ectotherms. Darker  
183 coloration is assumed to increase camouflage and protection against parasites. Whether these  
184 environmental factors simultaneously act on multiple colour signals with different production  
185 mechanism or independently influence specific colour signals remains an elusive question.

186 In this study, we used the common lizard (*Zootoca vivipara*) to shed light on the  
187 different factors explaining the variation of multiple colour signals. Male common lizards  
188 have a conspicuous, carotenoid-based ventral colouration that ranges from yellow to orange,  
189 with a varying amount of melanic black spots dispersed along the venter, and a conspicuous  
190 UV-white throat (Bonnaffé et al., 2018; Figure 1). The UV colouration on the throat plays a  
191 role during male-male competition (Martin et al., 2016) and female mate choice (Badiane et  
192 al., 2020), and is associated with social costs during male-male interactions (Kawamoto et al.,  
193 2021). Similarly, the amount of black ventral colouration correlates positively with male bite

194 force, but decreases with sprint speed (San-Jose et al., 2017). Finally, the carotenoid-based  
195 yellow-orange ventral colouration which covaries with the physiological stress response is  
196 maintained by frequency-dependent selection, and seems to play a role during female mate  
197 choice (Fitze et al., 2009; San-Jose & Fitze, 2013).

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201 **Figure 1.** Ventral photograph of an adult male common lizard (left). Reflectance spectra from the throat, the  
202 venter, and the dorsum of adult male common lizards between 300 and 700 nm, the typical visual range of  
203 common lizards (right). Lines represent the mean spectra from 211 individuals included in this study, and  
204 shaded areas represent the standard errors.

205

206 We explored the degree to which these three components of ventral colouration with three  
207 distinct production mechanisms covary with individual quality traits, parasite load and T  
208 levels among individuals. We also examined how these colour components varied along  
209 environmental gradients, including habitat structure (forest cover), climate variables  
210 (temperature and moisture), and geographical variables (elevation and latitude) among several  
211 wild populations. Our goals are to: i) explore how multiple colour signals relate to their

212 potential information content and associated costs, ii) examine the role of T as a mediator of  
213 these relationships, and iii) explore how environmental factors explain colour signal variation  
214 through their potential effect on signal efficacy and thermoregulation.

## 215 **Material & Methods**

### 216 *Study species and populations*

217 The European common lizard (*Zootoca vivipara*, family Lacertidae) is a small-sized species  
218 inhabiting cool and mesic habitats across Eurasia. This species is polygynandrous with both  
219 males and females mating with multiple partners (Fitze et al., 2005). We sampled adult male  
220 lizards in 13 populations that differ in several ecogeographical components across the Massif  
221 Central mountain range in France (Supp. Info. S1). We first recorded variables affecting  
222 habitat light conditions. We characterised the proportion of forest cover (mean = 33%, range  
223 = 0-90%) for each population using Google Earth Pro landscape orthophotographs (2020  
224 Google Image Landsat, Copernicus). Using the polygon tool, we surrounded each tree and  
225 group of trees from a given population and calculated the forested area in relation to the total  
226 population area. Forest cover is a direct index of habitat closeness, which can affect colour  
227 signal efficacy in lizards by modifying the ambient light environment (Endler, 1993; Leal &  
228 Fleishman, 2004). We also measured ambient light by taking irradiance spectra (i.e. 300-700  
229 nm) from 20 random points at 12:00 pm under clear sky within each population using a JAZ  
230 spectrophotometer mounted with a cosine corrected sensor (Ocean Optics Inc.). In our study,  
231 the degree of forest cover has a weak negative correlation with mean total irradiance (i.e. 300-  
232 700 nm;  $r = 0.52$ ,  $p < 0.001$ ), but a large, negative correlation with mean irradiance UV  
233 chroma (i.e. the proportion of UV in the irradiance spectrum  $I_{300-400}/I_{300-700}$ ;  $r = 0.95$ ,  $p <$   
234  $0.001$ ) since foliage absorbs UV light (Endler, 1993). We included two ecogeographical  
235 variables, elevation and latitude, that could affect overall climate conditions and thus impose  
236 thermoregulation constraints on lizards (Rozen- Rechels et al., 2021). We also recorded the

237 mean annual temperatures and calculated a pluviometric quotient (i.e.  $Q$  Emberger, a measure  
238 of moisture conditions) using the same methods as Rozen- Rechels et al. (2021) since both  
239 temperature and humidity are involved in thermoregulation (Rozen- Rechels et al., 2021).  
240 High values of the  $Q$  index indicate more mesic conditions.

#### 241 *Morphology and colouration*

242 In June 2018, we caught 211 adult males by hand from the 13 populations described above.  
243 We brought the lizards to a field laboratory and placed them in individual terraria for a  
244 maximum of 10 days. Individuals were given water *ad libitum* and live meal worms (*Tenebrio*  
245 *molitor*) every two days. On the day of capture, we weighed each lizard with a digital balance  
246 (to the nearest 0.01 g) and measured their snout-vent length (SVL in mm) with a ruler. We  
247 then obtained reflectance spectra from the belly and throat using a JAZ spectrophotometer  
248 (Ocean Optics Inc.) following the recommendations of Badiane et al. (2017) (Supp. Info. S2).  
249 To quantify the ventral black coloration, we took calibrated (colour standards, millimetre  
250 scale) photographs in raw format of each lizard's venter using a Nikon D5300 DSLR with a  
251 Nikkor 40 mm Micro lens. We then used ImageJ (Schneider, Rasband, & Eliceiri, 2012) to  
252 calculate the surface area covered by black colouration on the venter (i.e. absolute black), and  
253 the percentage of black colouration relative to the total ventral area (i.e. relative black).

#### 254 *Biomechanical performances*

255 The next day, we measured bite force, a measure of fighting ability and whole-organism  
256 performance in lizards (Lappin & Husak, 2005), using a purpose-built bite force meter  
257 constructed from a modified Sauter 25N digital force gauge. We retained the maximum score  
258 out of three repeated measurements. After two days of rest, we estimated their locomotor  
259 performance at two ecologically relevant tasks: maximum sprint speed and endurance  
260 capacity. To measure endurance, we induced lizards to run on a motorised treadmill, by  
261 gently tapping their hindlimbs, at the constant speed of 0.5 km/h until exhaustion. Exhaustion

262 was assessed when lizards did not respond to three hindlimbs taps and lost their righting  
263 response (ability to flip over when placed on their back). To measure sprint speed, we induced  
264 lizards to sprint down a 1.5-m racetrack lined with 25-cm splits, and recorded the trials with a  
265 high-speed camera (240 fps) placed above the racetrack. We analysed each trial frame by  
266 frame and retained the maximal sprint speed of the fastest 25-cm section using the program  
267 Tracker. For logistical reasons, we were able to measure sprint speed for only 108 of the 211  
268 individuals. Before each performance assay, we made sure the lizards had a body temperature  
269 close to 34°C during the tests (Rozen-Rechels et al., 2020). Rozen-Rechels et al. (2021) found  
270 minor differences in thermal preferences between our study populations (only 2% of the  
271 variance while inter-individual differences explained 24% of the variance) and we therefore  
272 used the same body temperature for performance tests across all populations.

### 273 *Plasma testosterone levels and parasitism*

274 We collected blood samples (40-60 µL) from the infra-orbital sinus using 20-µL  
275 microhematocrit tubes. We isolated the plasma by centrifugation and assayed plasma T levels  
276 using colorimetric competitive enzyme immunoassays (Testosterone ELISA kit, Cayman  
277 Chemical Cat, ref. 582701). On the day of capture, we also counted the number of ticks  
278 present on the body of each individual, an ectoparasite commonly found in common lizards  
279 (Wu et al., 2019). Ectoparasite infestation affects the life-history traits, locomotor  
280 performance, dispersal behaviour, and coloration of common lizards (Cote et al., 2010; Sorci  
281 & Clobert, 1995). We also quantified the prevalence of blood parasites from the  
282 haemogregarine group, which depends on environmental conditions (Álvarez-Ruiz et al.,  
283 2018) and can affect colour signalling, performance, and metabolism (Megía-Palma et al.,  
284 2016a; Molnár et al., 2013; Oppliger et al., 1996). To do so, we spread a drop of blood on a  
285 microscope slide and let it dry. We then fixated the slides into methanol for 60 s, let it dry,  
286 and immersed them in modified Giemsa stain (GS500, Sigma-Aldrich) for 45 min. Next, we

287 used a microscope (x400) to count the number of blood cells infested by an haemogregarine  
288 parasite on the basis of 10,000 blood cells counted per individual.

### 289 *Statistical analysis*

290 We processed spectral data in R v.3.6.2 (R Development Core Team, 2017) using the package  
291 *pavo* 2.0 (Maia et al., 2019). We extracted the chromatic variables UV chroma ( $R_{300-400}/R_{300-}$   
292  $700$ ) and luminance ( $R_{300-700}$ ) from the UV throat, and yellow-orange chroma ( $R_{575-700}/R_{300-700}$ )  
293 and luminance ( $R_{300-700}$ ) from the yellow-orange venter. To explore the relationships among  
294 colour traits, morphology, physiological performance, parasitism, and plasmatic T levels, we  
295 used piecewise structural equation modelling (SEM) using the *piecewiseSEM* v2.1 package in  
296 R (Lefcheck, 2016) in combination with generalised linear mixed models using the R  
297 packages *nlme* (Pinheiro et al., 2019) and *lme4* (Bates et al., 2015). SEM is a suitable tool to  
298 evaluate direct and indirect effects in descriptive analyses of complex systems (Grace et al.,  
299 2010). In addition, piecewise SEM tests for missing paths between variables using Shipley's  
300 test of d-separation, which allows us to adjust the initial model to improve its fit and  
301 biological significance. Assessment of the goodness-of-fit of a model is first indicated by a  
302 non-significant p-value based on a Chi-square test. Then, goodness-of-fit can be improved  
303 using a combination of indices, including Akaike's Information Criterion corrected for small  
304 sample size (AICc) obtained from Fisher's C statistic, and the Bayes-Schwarz Information  
305 Criterion (BIC).

306 We first built an initial model (detailed in Supp. Info. S3) with physiological  
307 performance (i.e. endurance, bite force) and parasitism (i.e. number of ticks, number of  
308 haemogregarines) having direct effects on colour variation (i.e. throat UV chroma and  
309 luminance, ventral yellow-orange chroma and luminance, black area) as these variables are  
310 often involved in condition-dependent signalling. We added a direct effect of body size on  
311 absolute black area based on the tests of d-separation. We further set body size and T levels as

312 direct drivers of the two physiological performance traits based on previous studies (Huyghe  
313 et al., 2009; John-Alder et al., 2009) and further added a direct effect of haemogregarines on  
314 bite force based on d-separation tests. We also tested a direct effect of T levels on body size  
315 (John-Alder et al., 2009) and parasitism (Fuxjager et al., 2011). In addition, we specified  
316 correlated errors between our five colour variables, between the number of ticks and  
317 haemogregarines, and between bite force and endurance. This allows the residual errors of  
318 two variables to be correlated for a reason not explained by our model when a direct causal  
319 effect is not biologically relevant. Both parasitism variables may reflect individual immunity  
320 and correlate with each other. Similarly, both performance variables may covary with other  
321 aspects of physiology and with each other.

322 We then discarded the non-significant predictors until we obtained the lowest values  
323 of BIC. This piecewise SEM was performed on a sample size of 134 lizards, because we  
324 could not perform the most invasive protocols (performance scoring and blood sampling) in  
325 two populations subject to a long-term mark-recapture survey and we had to exclude them  
326 from this part of the analysis (PIM and ROB in Supp. Info. S1). In addition, we ran the same  
327 piecewise SEM using the relative black area instead of the absolute black area to test whether  
328 results differed. Furthermore, sprint speed was measured only on a subset of individuals and  
329 was thus analysed separately using GLMMs with the five colour variables as response (in five  
330 separate models), sprint speed and body size as predictor, and population as random intercept.

331 To explore the effects of ecogeographical variables on lizard colouration across all  
332 populations, we analysed variation in UV chroma, yellow-orange chroma, throat luminance,  
333 ventral luminance, and absolute and relative area of black. We used GLMMs using the *lme*  
334 function in the R package *nlme* v3.1 (Pinheiro et al., 2019) with population identity as a  
335 random effect. We fitted a null model with only the intercept, and a model for every possible  
336 combination of the following predictors (only additive effects): elevation, latitude, forest

337 cover, pluviometric quotient  $Q$ , and  $T_{\text{mean}}$ . We then proceeded with a model selection based  
338 on AICc, selecting only the best models ( $\text{AICc} < 2$ ), and performed a conditional model  
339 averaging procedure using the R package MuMIn (Bartoń, 2019).

340 All continuous variables were mean-centred-scaled by the standard deviation prior to  
341 analysing to improve interpretation of the results (Schielzeth, 2010). We calculated a false  
342 discovery rate (FDR) of 15%, 20% and 25% following the Benjamini-Hochberg procedure to  
343 correct for multiple testing for both the SEM and model averaging analyses (Benjamini &  
344 Hochberg, 1995). All statistics of the SEM are summarised in Supp. Info. S4 with FDR  
345 information available in Supp. Info. S5. Analyses of ecophysiological variables are provided  
346 in Supp. Info. S6 with FDR information available in Supp. Info. S7.

#### 347 *Ethical approval*

348 To capture common lizards *Zootoca vivipara* and collect all the data described above, we  
349 obtained authorizations from the French ministry of Ecological Transition (ref  
350 TREL1734890A/6), the Cévennes National Parc (ref 2018-0133), and the Ethics Committee  
351 for Animal Experimentation (ref 14574 2018040913579349 v4).

## 352 **Results**

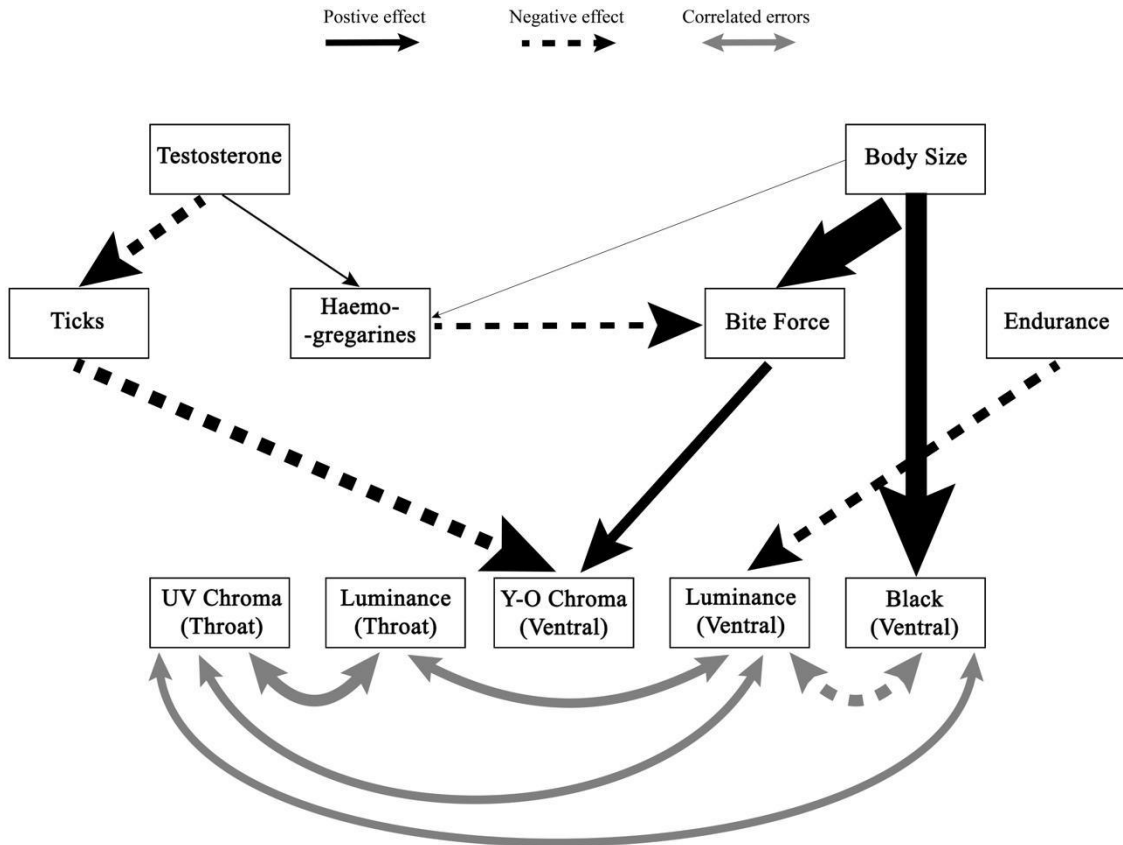
### 353 *Colour signals and information content*

354 The best model resulting from the piecewise SEM is presented in Figure 2. Our final model  
355 has a Fisher's C statistic of 32.135 with a p-value of 0.46 and 32 degrees of freedom,  
356 implying that C conforms to what is expected given the number of degrees of freedom and  
357 that the model provided a good fit to our data. According to this best model, the number of  
358 haemogregarines increased with increasing T levels and body size ( $R^2_{\text{m}} = 0.04$ ,  $R^2_{\text{c}} = 0.86$ ).  
359 In contrast, the number of ticks decreased with increasing T levels ( $R^2_{\text{m}} = 0.06$ ,  $R^2_{\text{c}} = 0.74$ ).  
360 In addition, bite force covaried with body size, but was negatively affected by the number of  
361 haemogregarine parasites ( $R^2_{\text{m}} = 0.59$ ,  $R^2_{\text{c}} = 0.62$ ). We found no direct relationship between



362 endurance and any other variables. Similarly, throat UV chroma is not influenced by bite  
 363 force, endurance, parasitism, or body size but correlates positively with both throat and  
 364 ventral luminance (correlated residual errors).

365



366

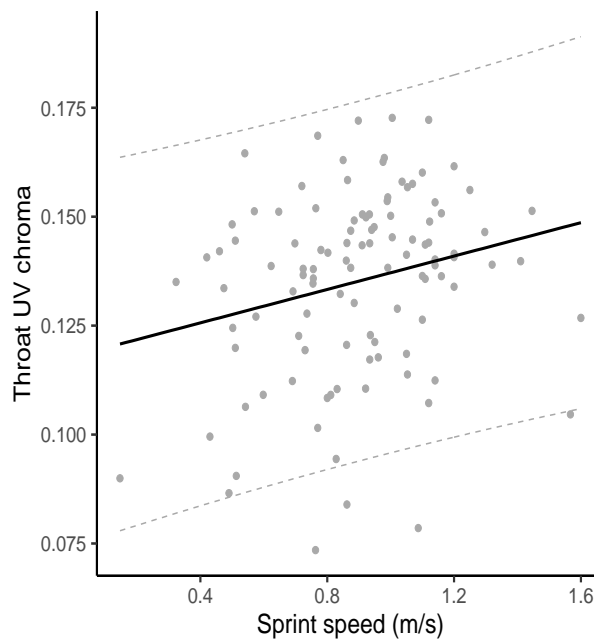
367 **Figure 2.** Best selected path diagram representing the relationships between colour signals (bottom line, 5 colour  
 368 components), body size (measured by snout-vent length), physiological performance (bite force and  
 369 endurance), parasitism (number of ticks and blood haemogregarines) and plasma testosterone levels. Each  
 370 single-headed arrow represents a statistically significant direct causal path and arrow thickness is  
 371 proportional to their effect size. Significant correlations are indicated by double header arrows.

372

373 Furthermore, yellow-orange chroma increases with bite force and decreases with tick  
 374 prevalence, but endurance does not have any significant influence ( $R^2_m = 0.16$ ,  $R^2_c = 0.17$ ).  
 375 Ventral luminance is negatively affected by endurance, but not by tick prevalence ( $R^2_m =$   
 376  $0.07$ ,  $R^2_c = 0.28$ ), and correlates positively with throat luminance and UV chroma, but

377 negatively with the amount of black colouration. Moreover, the amount of black colouration  
378 depends directly on body size ( $R^2_m = 0.21$ ,  $R^2_c = 0.29$ ) and correlates positively with throat  
379 UV chroma but negatively with ventral luminance. When we used the proportion of black  
380 colouration (%) instead of the absolute amount of black, the best model was qualitatively  
381 similar except that the proportion of black (%) is positively influenced by bite force instead of  
382 body size ( $\beta = 3.31 \pm 1.66$ , standardised  $\beta = 0.17$ ,  $p = 0.049$ ,  $R^2_m = 0.03$ ,  $R^2_c = 0.18$ ; Path  
383 diagram showed in Supp. Info. S8). In the sample of lizards measured for sprint speed, a  
384 separate LMM revealed that only throat UV chroma has a weak, positive correlation with  
385 sprint speed ( $\beta = 0.01 \pm 0.01$ ,  $p = 0.049$ ,  $R^2_m = 0.04$ ,  $R^2_c = 0.26$  – Figure 3).

386



387

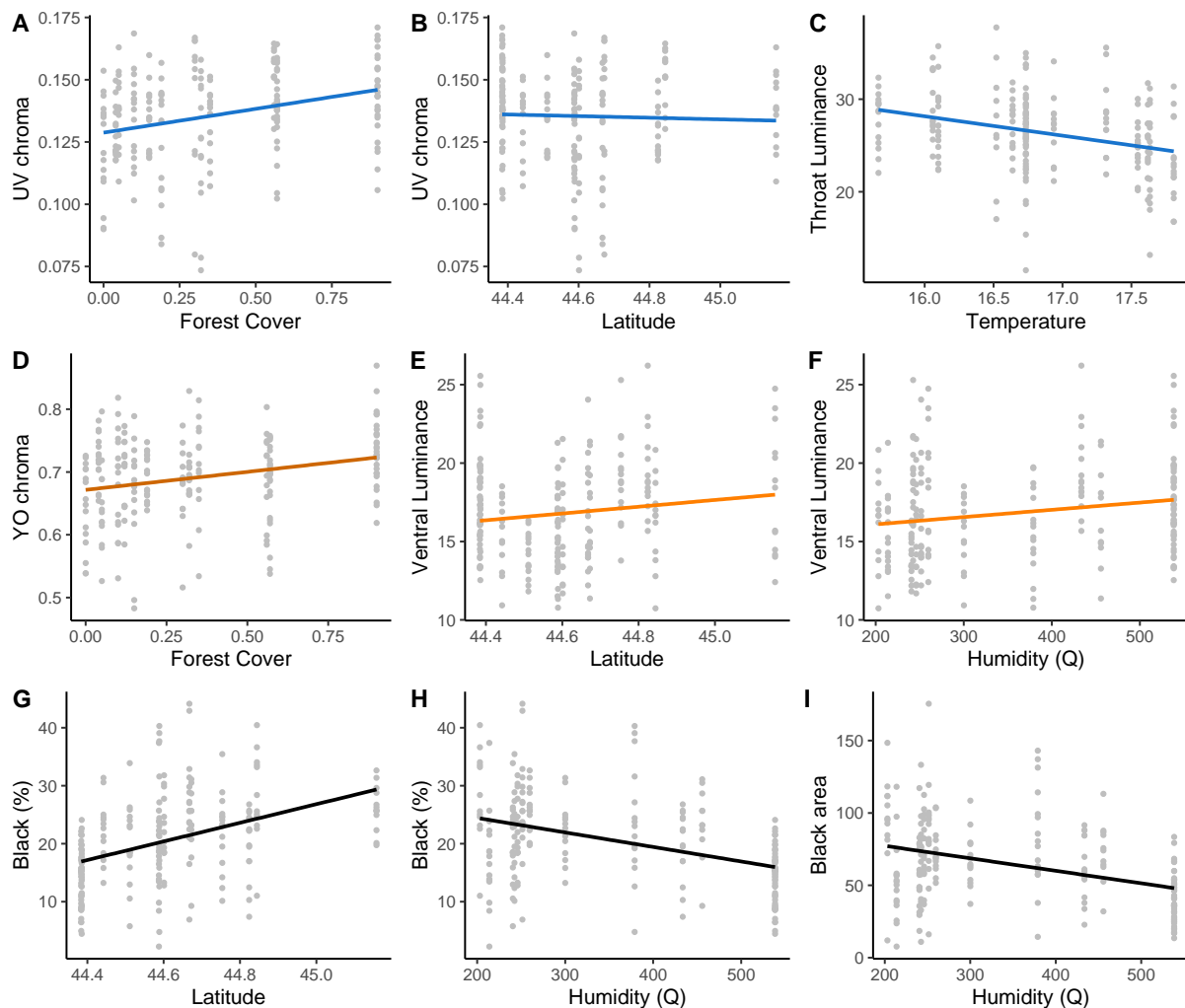
388 **Figure 3.** Best regression line between individual values of throat UV chroma (proportion of UV reflectance on  
389 the throat colour patch) and maximal sprint speed in male common lizards. Shaded area represents the 95%  
390 confidence interval and dashed lines represents the 95% prediction interval.

391

392 *Colour signals and ecogeographical variables*

393 Regarding the effects of ecogeographical variables on lizard colouration (summarised in  
 394 Table 1, Figure 4), in populations with higher forest cover, male common lizards have  
 395 significantly higher throat UV chroma and higher ventral yellow-orange chroma. In addition,  
 396 throat luminance decreases when mean annual temperatures increase, and ventral luminance  
 397 increases at higher latitude and in more mesic habitats (i.e. higher values of  $Q$ ). Finally, male  
 398 common lizards with higher absolute amounts of black colouration are found in less mesic  
 399 conditions. We also found this effect when we used relative black variable, which further  
 400 correlates positively with latitude.

401



402

403 **Figure 4.** Linear regressions (based on raw data) between colour signal components and eco-geographical  
 404 variables, namely throat UV chroma with forest cover (A) and latitude (B), throat luminance with mean annual

405 temperature (C), ventral yellow-orange chroma with forest cover (D), ventral luminance with latitude (E) and  
406 moisture (F), the percentage of black coloration with latitude (G) and moisture (H), and the absolute extent of  
407 black coloration with moisture (I). Blue, orange, and black regression lines refer the throat, belly and black  
408 coloration, respectively.

## 409 **Discussion**

410 Holistic studies integrating both environmental factors and individual traits as potential  
411 drivers of colour signal variation are scarce, and yet they have the potential to bring  
412 invaluable insights into the complexity of signalling systems. For example, Moreno-Rueda et  
413 al. (2021) showed that proxies of environmental conditions and individual quality predicted  
414 variation of two distinct colour signals in the lacertid lizard *Psammodromus algirus*, in  
415 directions comparable to our results. Here, we found that the three different colour signals  
416 covary with different aspects of male quality, thus supporting the ‘multiple message’  
417 hypothesis, and to a lesser degree the “backup” hypothesis (Johnstone, 1996). The yellow-  
418 orange ventral colouration correlates negatively with tick load, thus indicating good health,  
419 and positively with bite force, a performance trait involved in lizards’ male-male competitive  
420 interactions (e.g. Husak et al., 2006) and male sexual harassment towards females (Fitzé et al.,  
421 2005). In addition, the absolute and relative black colouration area increase with body size  
422 and bite force, respectively, confirming previous findings obtained in a separate, oviparous  
423 lineage of this species (San-Jose et al., 2017). Furthermore, the throat UV colouration of  
424 males correlated (albeit weakly based on values of the coefficient of determination) with  
425 maximal sprint speed, a trait important for fitness in lizards (Miles, 2004). This result is  
426 consistent with the role of UV signals previously identified in this species during male-male  
427 competition (Martin et al., 2016; Kawamoto et al., 2021) and female mate choice (Badiane et  
428 al., 2020). Our study thus indicates that receivers can extract information about multiple  
429 aspects of male condition including locomotor performance, bite force and parasite infestation  
430 from several components of the colouration of adult male common lizards.

431 **Table 1.** Results from our conditional model averaging procedures examining the effect of ecogeographical variables on lizard coloration for  
432 each colour variable (6 components). We provide the conditional averaged effect size estimates (standardised), standard errors, p-values, and  
433 importance value (i.e., sum of weights [sw]) associated with the effect of each ecogeographical predictor on each response colour variable.  
434 Statistically significant effects are in boldface and level of significance is indicated (\* < 0.05, \* < 0.01\*\*, < 0.001\*\*\*).

Colour variable	Elevation	Latitude	Forest Cover	Moisture	T <sub>mean</sub>
Throat UV chroma	-0.168 ± 0.097 p = 0.140 sw = 0.65	<b>0.212 ± 0.087</b> <b>p = 0.039 *</b> <b>sw = 0.85</b>	<b>0.449 ± 0.107</b> <b>p &lt; 0.001 ***</b> <b>sw = 0.99</b>	0.017 ± 0.109 p = 0.891 sw = 0.28	-0.032 ± 0.111 p = 0.803 sw = 0.31
Throat luminance	-0.124 ± 0.106 p = 0.312 sw = 0.62	0.141 ± 0.085 p = 0.146 sw = 0.43	0.108 ± 0.099 p = 0.332 sw = 0.43	-0.005 ± 0.096 p = 0.965 sw = 0.28	<b>-0.339 ± 0.091</b> <b>p = 0.001 ***</b> <b>sw = 1.00</b>
Ventral Y-O chroma	0.122 ± 0.125 p = 0.377 sw = 0.41	-0.085 ± 0.100 p = 0.449 sw = 0.35	<b>0.223 ± 0.094</b> <b>p = 0.038 *</b> <b>sw = 0.77</b>	-0.086 ± 0.110 p = 0.490 sw = 0.36	-0.187 ± 0.087 p = 0.061 sw = 0.72
Ventral luminance	0.212 ± 0.112 p = 0.099 sw = 0.42	<b>0.294 ± 0.106</b> <b>p = 0.014 *</b> <b>sw = 0.91</b>	-0.056 ± 0.133 p = 0.712 sw = 0.29	<b>0.275 ± 0.121</b> <b>p = 0.045 *</b> <b>sw = 0.84</b>	-0.127 ± 0.124 p = 0.360 sw = 0.71
Black (absolute)	0.175 ± 0.144 p = 0.289 sw = 0.44	0.216 ± 0.136 p = 0.161 sw = 0.55	-0.132 ± 0.168 p = 0.488 sw = 0.33	<b>-0.340 ± 0.154</b> <b>p = 0.049 *</b> <b>sw = 0.76</b>	-0.072 ± 0.147 p = 0.665 sw = 0.31
Black (%)	0.106 ± 0.132 p = 0.483 sw = 0.35	<b>0.280 ± 0.121</b> <b>p = 0.040 *</b> <b>sw = 0.81</b>	-0.075 ± 0.155 p = 0.669 sw = 0.29	<b>-0.311 ± 0.139</b> <b>p = 0.047 *</b> <b>sw = 0.78</b>	-0.104 ± 0.122 p = 0.453 sw = 0.36

435

436

437           Contrary to our initial predictions, T does not seem play a central role in colour signal  
438 variation as it had no direct effect on colouration, but influenced colour signal variation only  
439 indirectly through its effects on parasitism. We found a dual effect of T levels on parasite  
440 load, decreasing tick prevalence while increasing haemogregarines prevalence at the same  
441 time. Fuxjager et al. (2011) also found contrasting effects of plasma T levels on parasitism in  
442 the lizard *Sceloporus jarrovi*, but with a different pattern since it increased mite load,  
443 decreased gastrointestinal nematodes, and had no effect on blood parasites. Some studies  
444 showed a positive association between T and parasite load (e.g. Olsson et al., 2000) while  
445 others found no correlations at all (e.g. Oppliger et al., 2004). Therefore, it seems that the  
446 relationship between T levels, parasitism and immunity is more complex than proposed by the  
447 ICHH (Folstad & Karter, 1992). It may be because the relationship between T and the  
448 immune system can be complex in itself, for example depressing only adaptive immunity  
449 while reinforcing innate immunity (Ezenwa et al., 2012). Also, T can affect parasitism  
450 through its independent effect on behaviour, for example increasing male sexual activity  
451 which might favour infestation by ectoparasites but not necessarily by endoparasites (e.g.  
452 Olsson et al., 2000). Finally, parasite load may be explained by other factors than T such as  
453 environmental conditions. For example, Wu et al. (2019) showed that, in common lizards,  
454 tick infestation decreased with elevation and vegetation cover, and increased with human  
455 disturbances and the presence of livestock.

456           In addition, tick prevalence had a negative effect on the yellow-orange signals while  
457 haemogregarine prevalence directly reduced male performance, which in turn reduced  
458 yellow-orange chroma. This phenotypic correlation is superficially consistent with the  
459 predictions of the Hamilton-Zuk hypothesis (Hamilton and Zuk, 1982), which assumes an  
460 association between bright colour signals and low parasite load. However, exploring the  
461 genetic mechanisms involved would be necessary to draw a firm conclusion because

462 Hamilton-Zuk hypothesis posits a genetic correlation between sexual ornaments and  
463 resistance to parasites (Balenger & Zuk, 2014). In contrast, parasite load was uncorrelated  
464 with UV colouration, diverging from previous results showing either positive or negative  
465 correlations between parasite load and UV signals in other lacertid species (e.g. Megía-Palma  
466 et al., 2016a, b).

467         Our results further indicate that high endurance reduces yellow-orange luminance.  
468 Previous evidence in lacertid lizards, including in our species, suggest that a high  
469 concentration of carotenoid pigments in the skin reduces luminance (Megía-Palma et al.,  
470 2021) and produces red-shifted hues (Fitze et al., 2009). In light of this, our findings may  
471 reflect an effect of endurance on hue, which in this case ranges from yellow to orange.  
472 Because the reflectance peak of a yellow hue is located at lower wavelengths than for an  
473 orange hue, the total luminance of orange colours is generally lower than for yellow. This  
474 would mean that males with orange-shifted hues have higher endurance capacities than males  
475 with yellow-shifted hues (Sinervo et al., 2007), which would make sense because male  
476 common lizards gradually turn from yellow to orange as they age (Bonnaffé et al., 2018).  
477 Alternatively, a less parsimonious hypothesis may posit that a high endurance capacity  
478 constrains yellow-orange luminance. The carotenoid-based colouration of common lizards  
479 appears to be correlated with tocopherols (i.e., vitamin E) availability and dietary lipids such  
480 that high dietary lipid uptake reduces vitamin E circulating levels, which in turn reduces  
481 carotenoid-based colour expression (San-Jose et al., 2012). Thus, we could speculate that a  
482 high endurance requires a high dietary lipid uptake, therefore constraining yellow-orange  
483 luminance (San-Jose et al., 2012). Another explanation may rely on previous evidence  
484 showing that iridophores, and not carotenoids, can account for the variation of the yellow-  
485 orange ventral colouration of common lizards (San-Jose et al., 2013), in which case the above

486 hypotheses would not be plausible. In any case, studies investigating the physiological basis  
487 of the relationship between this colour trait and endurance are needed to test these hypotheses.  
488  
489 For a signal to be successful, not only should it make honest information available to the  
490 receivers, but it must also transmit well through the environment towards the receiver  
491 (Bradbury & Vehrencamp, 2011). Variation in colour signals therefore reflect to some extent  
492 variation in the environmental light conditions, which depend on factors such as habitat  
493 structure and climatic conditions, so as to maximise signal detectability (Fleishman et al.,  
494 2020). Here, we first showed that the degree of forest cover negatively impacted the amount  
495 of average incoming light (i.e. irradiance), and especially in the UV range. Interestingly, both  
496 UV and yellow-orange signals of male lizards increased in chroma in populations with higher  
497 forest cover, that is in habitats characterised by degraded ambient light conditions. We  
498 suggest that these highly saturated colour signals are favoured by natural selection in these  
499 habitats to compensate for reduced light conditions and increase signal detectability. Along  
500 the same lines, Leal and Fleishman (2004) found that the lizard *Anolis cristatellus* had a  
501 brighter dewlap in mesic forests and a darker dewlap in xeric forests, which in both cases  
502 increased dewlap detectability. Comparable correlations between signal features and  
503 environmental parameters enhancing signal detectability have been shown in other taxa such  
504 as birds and fishes, and with other signalling modes such as acoustic and chemical signals  
505 (see Cummings & Endler, 2018 and references therein).

506         We also found that the three types of colour signals co-varied with latitude and local  
507 climatic conditions. Surprisingly, these geographic gradients do not support, and even oppose,  
508 the predictions from the thermal melanism hypothesis (Clusella Trullas et al., 2007), and from  
509 the revised version of the “Gloger’s rule” (Delhey, 2019) stating that animals are darker in  
510 humid environments (although the latter seems to apply to endothermic animals only). With



511 the exception of the relative amount of black colouration, we indeed found that lizards from  
512 populations at higher latitudes, in more humid conditions and with lower temperatures had  
513 generally brighter throat and belly colouration with fewer black spots (Table 1, Figure 4).  
514 This unexpected finding suggests that mesic conditions promote generally brighter colour  
515 signals. Our results allow us to propose several hypotheses. First, more mesic habitats usually  
516 have higher cloud cover on average and denser vegetation, thus degrading ambient light  
517 conditions (e.g. Thorpe, 2002). Therefore, selection on signal efficacy may increase overall  
518 signal conspicuousness to optimise its detectability. Second, these mesic conditions may offer  
519 more favourable conditions, for example by relaxing potential constraints such as predation  
520 and parasitism, or by providing more resources, allowing common lizards to increase  
521 investment in sexual signals. For instance, the biology of this species is highly dependent on  
522 access to water, a resource that may be more abundant in more mesic conditions (see Rozen-  
523 Rechels et al., 2021 and references therein). Altogether, these analyses suggest that habitat  
524 constraints on signal efficacy and climatic conditions explain colour signal variation across  
525 populations in this species, even though ca. 80% of the variance is left unexplained in our  
526 model.

527

528 In summary, our study shows that multiple colour signals with different production  
529 mechanisms vary in how they relate to their information content and to the local light  
530 conditions. The three colour signals indeed exhibit condition-dependent variation, involving  
531 performance, morphology and parasitism, suggesting that they convey honest information  
532 about different aspects of male condition. However, despite its effect on parasitism, T does  
533 not seem to play a central role as a mediator between colour signals and their information  
534 content, thus contradicting the immunocompetence handicap hypothesis (Folstad & Karter,  
535 1992). At the same time, our results suggest that both UV and yellow-orange signals respond

536 to variations in local light conditions so as to increase signal efficacy, and that all three colour  
537 signals respond to climatic conditions in a direction ruling out thermal melanism. Our  
538 integrative study demonstrates that individual quality traits and environmental conditions  
539 simultaneously explain the variations of multiple colour signals, and that the nature of these  
540 relationships is conditional on colour production mode.

541

#### 542 **Data accessibility**

543 Data and code used in this study are freely available from the public repository Zenodo  
544 <https://doi.org/10.5281/zenodo.6683661> (Badiane et al., 2022)

545

546

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553

#### 554 **Conflict of interest**

555 The authors have no conflict of interest to declare

556

#### 557 **Authors contributions**

558 Badiane and Le Galliard conceived the study. Badiane, Dupoué, Blaimont, Miles, Guilbert,  
559 and Clobert performed fieldwork and collected the raw data. Badiane, Leroux-Coyau,  
560 Kawamoto, and Rozen-Rechels processed the data. Badiane performed the statistical

561 analyses. Le Galliard and Meylan supervised and commented each step of the process.  
562 Badiane and Le Galliard wrote the manuscript and all authors provided invaluable feedback  
563 on the manuscript.

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## 568 **References**

569 Álvarez-Ruiz, L., Megía-Palma, R., Reguera, S., Ruiz, S., Zamora-Camacho, F. J., Figuerola,  
570 J., & Moreno-Rueda, G. (2018). Opposed elevational variation in prevalence and  
571 intensity of endoparasites and their vectors in a lizard. *Current Zoology*, *64*, 197-204.  
572 doi: 10.1093/cz/zoy002

573 Andrade, P., & Carneiro, M. (2021). Pterin-based pigmentation in animals. *Biology Letters*,  
574 *17*, 20210221. doi: 10.1098/rsbl.2021.0221

575 Badiane, A., Dupoué, A., Blaimont, P., Miles, D.B., Gilbert, A., Leroux-Coyau, M.,  
576 Kawamoto, A., Rozen-Rechels, D., Meylan, S., Clobert, J., Le Galliard, J.F. (2022).  
577 Dataset: Environmental conditions and male quality traits simultaneously explain  
578 variation of multiple colour signals in male lizards. Zenodo,  
579 <https://doi.org/10.5281/zenodo.6683661>

580 Badiane, A., Martin, M., Meylan, S., Richard, M., Decencièrre Ferrandière, B., & Le Galliard,  
581 J.-F. (2020). Male ultraviolet reflectance and female mating history influence female  
582 mate choice and male mating success in a polyandrous lizard. *Biological Journal of the*  
583 *Linnean Society*, *130*, 586–598. doi: 10.1093/biolinnean/blaa061

584 Badiane, A., Pérez i de Lanuza, G., García- Custodio, M. del C., Carazo, P., & Font, E.  
585 (2017). Colour patch size and measurement error using reflectance spectrophotometry.

586 *Methods in Ecology and Evolution*, 8, 1585–1593. doi: 10.1111/2041-210X.12801

587 Balenger, S. L., & Zuk, M. (2014). Testing the Hamilton-Zuk hypothesis: Past, present, and  
588 future. *Integrative and Comparative Biology*, 54, 601–613. doi: 10.1093/icb/icu059

589 Bartoń, K. (2019). “MuMin”: Multi-model inference. R package version 1.43.6. Retrieved  
590 from <https://cran.r-project.org/package=MuMIn>

591 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models  
592 using lme4. *Journal of Statistical Software*, 67, 1–48.

593 Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate : A Practical  
594 and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*  
595 *Series B*, 57, 289–300.

596 Bonnaffé, W., Martin, M., Mugabo, M., Meylan, S., & Le Galliard, J. (2018). Ontogenetic  
597 trajectories of body coloration reveal its function as a multicomponent nonsenescent  
598 signal. *Ecology and Evolution*, 8, ece3.4369. doi: 10.1002/ece3.4369

599 Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication* (2nd ed.).  
600 Sinauer Association Press Sunderland.

601 Bro-Jørgensen, J. 2010. Dynamics of multiple signalling systems: animal communication in a  
602 world in flux. *Trends in Ecology and Evolution*, 25, 292-300

603 Clusella Trullas, S., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms.  
604 *Journal of Thermal Biology*, 32, 235–245. doi: 10.1016/j.jtherbio.2007.01.013

605 Cote, J., Meylan, S., Clobert, J., & Voituren, Y. (2010). Carotenoid-based coloration,  
606 oxidative stress and corticosterone in common lizards. *Journal of Experimental Biology*,  
607 213, 2116–2124. doi: 10.1242/jeb.040220

608 Cox, R. M., Stenquist, D. S., & Calsbeek, R. (2009). Testosterone, growth and the evolution  
609 of sexual size dimorphism. *Journal of Evolutionary Biology*, 22(8), 1586–1598. doi:  
610 10.1111/j.1420-9101.2009.01772.x

611 Cummings, M. E., & Endler, J. A. (2018). 25 Years of sensory drive: The evidence and its  
612 watery bias. *Current Zoology*, *64*, 471–484. doi: 10.1093/cz/zoy043

613 Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., ... Caro, T.  
614 (2017). The biology of color. *Science*, *357*, eaan0221. doi: 10.1126/science.aan0221

615 de Souza, A. R., Turillazzi, S., Lino-Neto, J., & Santini, G. (2016). Colder environments may  
616 select for darker paper wasps. *Biological Journal of the Linnean Society*, *120*, 700–704.  
617 doi: 10.1111/bij.12910

618 Delhey, K. (2019). A review of Gloger’s rule, an ecogeographical rule of colour: definitions,  
619 interpretations and evidence. *Biological Reviews*, *94*, 1294–1316. doi:  
620 10.1111/brv.12503

621 Endler, J. A. (1993). The color of light in forests and its implications. *Ecological*  
622 *Monographs*, *63*, 1–27. doi: 10.2307/2937121

623 Endler, J. A., and J. Mappes. 2017. The current and future state of animal coloration research.  
624 *Philosophical Transactions of the Royal Society B*, *372*, 20160352.

625 Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations  
626 between testosterone and parasite infection in the wild. *Functional Ecology*, *26*, 123–  
627 133. doi: 10.1111/j.1365-2435.2011.01919.x

628 Fitze, P. S., Cote, J., San-Jose, L. M., Meylan, S., Isaksson, C., Andersson, S., ... Clobert, J.  
629 (2009). Carotenoid-based colours reflect the stress response in the common lizard. *PLoS*  
630 *ONE*, *4*, e5111. doi: 10.1371/journal.pone.0005111

631 Fitze, P. S., Le Galliard, J.-F., Federici, P., Richard, M., & Clobert, J. (2005). Conflict over  
632 multiple-partner mating between males and females of the polygynandrous common  
633 lizards. *Evolution*, *59*, 2451–2459. doi: 10.1554/05-208.1

634 Fleishman, L. J., Wadman, C. S., & Maximov, K. J. (2020). The interacting effects of total  
635 light intensity and chromatic contrast on visual signal visibility in an *Anolis* lizard.

636 *Animal Behaviour*, 167, 263–273. doi: 10.1016/j.anbehav.2020.07.008

637 Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence  
638 handicap. *The American Naturalist*, 139, 603–622.

639 Foo, Y. Z., Nakagawa, S., Rhodes, G., & Simmons, L. W. (2017). The effects of sex  
640 hormones on immune function: a meta-analysis. *Biological Reviews*, 92, 551–571. doi:  
641 10.1111/brv.12243

642 Fuxjager, M. J., Foufopoulos, J., Diaz-Uriarte, R., & Marler, C. A. (2011). Functionally  
643 opposing effects of testosterone on two different types of parasite: Implications for the  
644 immunocompetence handicap hypothesis. *Functional Ecology*, 25, 132–138. doi:  
645 10.1111/j.1365-2435.2010.01784.x

646 Grace, J. B., Anderson, T. M., Olf, H., & Scheiner, S. M. (2010). On the specification of  
647 structural equation models for ecological systems. *Ecological Monographs*, 80, 67–87.  
648 doi: 10.1890/09-0464.1

649 Grether, G. F., Kolluru, G. R., and Nersissian, K. (2004). Individual colour patches as  
650 multicomponent signals. *Biological Reviews*. 79, 583–610.

651 Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for  
652 parasites? *Science*, 218, 384–387.

653 Hebets, E. A., and D. R. Papaj. 2005. Complex signal function: Developing a framework of  
654 testable hypotheses. *Behavioral Ecology and Sociobiology*, 57, 197–214.

655 Husak, J. F., Irschick, D. J., Meyers, J. J., Lailvaux, S. P., & Moore, I. T. (2007). Hormones,  
656 sexual signals, and performance of green anole lizards (*Anolis carolinensis*). *Hormones  
657 and Behavior*, 52, 360–367. doi: 10.1016/j.yhbeh.2007.05.014

658 Husak, J. F., Lappin, K. A., Fox, S. F., & Lemos-Espinal, J. A. (2006). Bite-force  
659 performance predicts dominance in male venerable collared lizards (*Crotaphytus  
660 antiquus*). *Copeia*, 2006, 301–306

661 Huyghe, Katleen, Husak, J. F., Herrel, A., Tadic, Z., Moore, I. T., Van Damme, R., &  
662 Vanhooydonck, B. (2009). Relationships between hormones, physiological performance  
663 and immunocompetence in a color-polymorphic lizard species, *Podarcis melisellensis*.  
664 *Hormones and Behavior*, 55, 488–494. doi: 10.1016/j.yhbeh.2009.02.005

665 John-Alder, H. B., Cox, R. M., Haenel, G. J., & Smith, L. C. (2009). Hormones, performance  
666 and fitness: Natural history and endocrine experiments on a lizard (*Sceloporus*  
667 *undulatus*). *Integrative and Comparative Biology*, 49, 393–407. doi: 10.1093/icb/icp060

668 Johnstone, R. A. (1996). Multiple displays in animal communication: 'backup signals' and  
669 "multiple messages." *Philosophical Transactions of the Royal Society B: Biological*  
670 *Sciences*, 351, 329–338. doi: 10.1098/rstb.1996.0026

671 Kawamoto, A., Le Galliard, J., & Badiane, A. (2021). The role of social costs as a mechanism  
672 enforcing the honesty of ultraviolet-reflecting signals in a lizard. *Biological Journal of*  
673 *the Linnean Society*, 133, 1126–1138. doi: 10.1093/biolinnean/blab008

674 Kemp, D. J., Herberstein, M. E., & Grether, G. F. (2012). Unraveling the true complexity of  
675 costly color signaling. *Behavioral Ecology*, 23, 233–236. doi: 10.1093/beheco/arr153

676 Khalil, S., Welklin, J. F., McGraw, K. J., Boersma, J., Schwabl, H., Webster, M. S., &  
677 Karubian, J. (2020). Testosterone regulates CYP2J19-linked carotenoid signal  
678 expression in male red-backed fairywrens (*Malurus melanocephalus*). *Proceedings.*  
679 *Biological Sciences*, 287, 20201687. doi: 10.1098/rspb.2020.1687

680 Kobayashi, M., Tajima, C., & Sugimoto, M. (2009). Effects of androgens on the development  
681 of nuptial coloration and chromatophores in the bitterling *rhodeus ocellatus ocellatus*.  
682 *Zoological Science*, 26, 125–130. doi: 10.2108/zsj.26.125

683 Lappin, A. K., & Husak, J. F. (2005). Weapon performance, not size, determines mating  
684 success and potential reproductive output in the collared lizard (*Crotaphytus collaris*).  
685 *American Naturalist*, 166, 426–436.

686 Leal, M., & Fleishman, L. J. (2004). Differences in visual signal design and detectability  
687 between allopatric populations of *Anolis* lizards. *The American Naturalist*, *163*, 26–39.  
688 doi: 10.1086/379794

689 Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for  
690 ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*, 573–579. doi:  
691 10.1111/2041-210X.12512

692 Lindstedt, C., Lindström, L., & Mappes, J. (2009). Thermoregulation constrains effective  
693 warning signal expression. *Evolution*, *63*, 469–478. doi: 10.1111/j.1558-  
694 5646.2008.00561.x

695 Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2019). *pavo 2*: New tools for the spectral  
696 and spatial analysis of colour in r. *Methods in Ecology and Evolution*, *10*, 1097–1107.  
697 doi: 10.1111/2041-210X.13174

698 Martín, J., & López, P. (2009). Multiple color signals may reveal multiple messages in male  
699 Schreiber’s green lizards, *Lacerta schreiberi*. *Behavioral Ecology and Sociobiology*, *63*,  
700 1743–1755. doi: 10.1007/s00265-009-0794-6

701 Martin, M., Meylan, S., Haussy, C., Decencière, B., Perret, S., & Le Galliard, J.-F. (2016).  
702 UV color determines the issue of conflicts but does not covary with individual quality in  
703 a lizard. *Behavioral Ecology*, *27*, 262–270. doi: 10.1093/beheco/arv149

704 Megía-Palma, R., Barrientos, R., Gallardo, M., Martínez, J., & Merino, S. (2021). Brighter is  
705 darker: the Hamilton-Zuk hypothesis revisited in lizards. *Biological Journal of the*  
706 *Linnean Society*, *in press*. doi: 10.1093/biolinnean/blab081/6310264

707 Megía-Palma, R., Martínez, J., & Merino, S. (2016a). Structural- and carotenoid-based throat  
708 colour patches in males of *Lacerta schreiberi* reflect different parasitic diseases. Megía-  
709 Palma, R., Martínez, J., & Merino, S. (2016b). A structural colour ornament correlates  
710 positively with parasite load and body condition in an insular lizard species. *Science of*



711 *Nature*, 103, 52. doi: 10.1007/s00114-016-1378-8

712 *Behavioral Ecology and Sociobiology*, 70, 2017–2025. doi: 10.1007/s00265-016-2205-0

713 Miles, D. B. (2004). The race goes to the swift: Fitness consequences of variation in sprint  
714 performance in juvenile lizards. *Evolutionary Ecology Research*, 6, 63–75.

715 Molnár, O., Bajer, K., Mészáros, B., Torok, J., & Herczeg, G. (2013). Negative correlation  
716 between nuptial throat colour and blood parasite load in male European green lizards  
717 supports the Hamilton-Zuk hypothesis. *Naturwissenschaften*, 100, 551–558. doi:  
718 10.1007/s00114-013-1051-4

719 Moreno-Rueda, G, Reguera, S, Zamora-Camacho, F. J., Comas, M (2021): Inter-individual  
720 differences in ornamental colouration in a Mediterranean lizard in relation to altitude,  
721 season, sex, age, and body traits. *Diversity* 13, 158. doi: 10.3390/d13040158

722 Olsson, M., Wapstra, E., Madsen, T., & Silverin, B. (2000). Testosterone, ticks and travels: a  
723 test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards.  
724 *Proceedings of The Royal Society London B*, 267, 2339–2343. doi:  
725 10.1098/rspb.2000.1289

726 Oppliger, A, Giorgi, M. S., Conelli, A., Nembrini, M., & John-Alder, H. B. (2004). Effect of  
727 testosterone on immunocompetence, parasite load, and metabolism in the common wall  
728 lizard (*Podarcis muralis*). *Canadian Journal of Zoology*, 82, 1713–1719. doi:  
729 10.1139/z04-152

730 Oppliger, Célérier, M. L., & Clobert, J. (1996). Physiological and behaviour changes in  
731 common lizards parasitized by haemogregarines. *Parasitology*, 113, 433–438.

732 Pérez i de Lanuza, G., Carazo, P., & Font, E. (2014). Colours of quality: Structural (but not  
733 pigment) coloration informs about male quality in a polychromatic lizard. *Animal*  
734 *Behaviour*, 90, 73–81. doi: 10.1016/j.anbehav.2014.01.017

735 Peters, A. (2007). Testosterone and carotenoids: an integrated view of trade-offs between

736 immunity and sexual signalling. *BioEssays*, 29, 427–430. doi: 10.1002/bies.20563

737 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). *nlme: Linear and*  
738 *nonlinear mixed effects models*. R package version 3.1-141.

739 Plasman, M., Reynoso, V. H., Nicolás, L., & Torres, R. (2015). Multiple colour traits signal  
740 performance and immune response in the Dickerson’s collared lizard *Crotaphytus*  
741 *dickersonae*. *Behavioral Ecology and Sociobiology*, 69, 765–775. doi: 10.1007/s00265-  
742 015-1892-2

743 Quinn, V. S., & Hews, D. K. (2003). Positive relationship between abdominal coloration and  
744 dermal melanin density in Phrynosomatid lizards. *Copeia*, 4, 858–864. doi:  
745 10.1643/h202-116.1

746 R Development Core Team. (2017). *R: A language and environment for statistical computing*.  
747 Vienna, Austria: R Foundation for Statistical Computing.

748 Reguera, S., Zamora-Camacho, F. J., & Moreno-Rueda, G. (2014). The lizard  
749 *Psammodromus algirus* (Squamata: Lacertidae) is darker at high altitudes. *Biological*  
750 *Journal of the Linnean Society*, 112, 132–141. doi: 10.1111/bij.12250

751 Roulin, A. (2016). Condition-dependence, pleiotropy and the handicap principle of sexual  
752 selection in melanin-based colouration. *Biological Reviews*, 91, 328–348. doi:  
753 10.1111/brv.12171

754 Rozen-Rechels, D., Badiane, A., Agostini, S., Meylan, S., & Le Galliard, J. (2020). Water  
755 restriction induces behavioral fight but impairs thermoregulation in a dry- skinned  
756 ectotherm. *Oikos*, 129, 572–584. doi: 10.1111/oik.06910

757 Rozen- Rechels, D., Rutschmann, A., DupouÉ, A., Blaimont, P., Chauveau, V., Miles, D. B.,  
758 ... Le Galliard, J. (2021). Interaction of hydric and thermal conditions drive geographic  
759 variation in thermoregulation in a widespread lizard. *Ecological Monographs*, 91, 1–19.  
760 doi: 10.1002/ecm.1440

761 San-Jose, L. M., & Fitze, P. S. (2013). Corticosterone regulates multiple colour traits in  
762 *Lacerta* [*Zootoca*] *vivipara* males. *Journal of Evolutionary Biology*, *26*, 2681–2690. doi:  
763 10.1111/jeb.12265

764 San-Jose, L. M., Granado-Lorencio, F., & Fitze, P. S. (2012). Dietary lipids reduce the  
765 expression of carotenoid-based coloration in *Lacerta vivipara*. *Functional Ecology*, *26*,  
766 646–656. doi: 10.1111/j.1365-2435.2012.01970.x

767 San-Jose, L. M., Granado-Lorencio, F., Sinervo, B., & Fitze, P. S. (2013). Iridophores and not  
768 carotenoids account for chromatic variation of carotenoid-based coloration in common  
769 lizards (*Lacerta vivipara*). *The American Naturalist*, *181*, 396–409. doi: 10.1086/669159

770 San-Jose, L. M., Huyghe, K., Schuerch, J., & Fitze, P. S. (2017). More melanized males bite  
771 stronger but run slower: potential performance trade-offs related to melanin-based  
772 coloration. *Biological Journal of the Linnean Society*, *122*, 184–196. doi:  
773 10.1093/biolinnean/blx045

774 Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients.  
775 *Methods in Ecology and Evolution*, *1*, 103–113. doi: 10.1111/j.2041-210X.2010.00012.x

776 Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH to ImageJ: 25 years of image  
777 analysis. *Nature Methods*, *9*, 671–675.

778 Shawkey, M. D., & D’Alba, L. (2017). Interactions between colour-producing mechanisms  
779 and their effects on the integumentary colour palette. *Philosophical Transactions of the*  
780 *Royal Society B: Biological Sciences*, *372*, 20160536. doi: 10.1098/rstb.2016.0536

781 Sinervo, B., Heulin, B., Surget-Groba, Y., Clobert, J., Miles, D. B., Corl, A., ... Davis, A.  
782 (2007). Models of density-dependent genic selection and a new rock-paper-scissors  
783 social system. *American Naturalist*, *170*, 663–680. doi: 10.1086/522092

784 Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M., & DeNardo, D. F. (2000).  
785 Testosterone, endurance, and Darwinian fitness: Natural and sexual selection on the

786 physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and*  
787 *Behavior*, 38, 222–233. doi: 10.1006/hbeh.2000.1622

788 Sorci, G., & Clobert, J. (1995). Effects of maternal parasite load on offspring life- history  
789 traits in the common lizard (*Lacerta vivipara*). *Journal of Evolutionary Biology*, 8, 711–  
790 723. doi: 10.1046/j.1420-9101.1995.8060711.x

791 Stuart-Fox, D. M., & Moussalli, A. (2009). Camouflage, communication and  
792 thermoregulation: lessons from colour changing organisms. *Philosophical Transactions*  
793 *of the Royal Society of London. Series B, Biological Sciences*, 364, 463–470. doi:  
794 10.1098/rstb.2008.0254

795 Svensson, E. I., & Waller, J. T. (2013). Ecology and sexual selection: Evolution of wing  
796 pigmentation in calopterygid damselflies in relation to latitude, sexual dimorphism, and  
797 speciation. *The American Naturalist*, 182, E174–E195. doi: 10.1086/673206

798 Svensson, P. A., & Wong, B. B. M. (2011). Carotenoid-based signals in behavioural ecology:  
799 A review. *Behaviour*, 148, 131–189. doi: 10.1163/000579510X548673

800 Thorpe, R. S. (2002). Analysis of color spectra in comparative evolutionary Studies:  
801 Molecular phylogeny and habitat adaptation in the St. Vincent anole (*Anolis trinitatis*).  
802 *Systematic Biology*, 51, 554–569. doi: 10.1080/10635150290069986

803 Wu, Q., Richard, M., Rutschmann, A., Miles, D. B., & Clobert, J. (2019). Environmental  
804 variation mediates the prevalence and co-occurrence of parasites in the common lizard,  
805 *Zootoca vivipara*. *BMC Ecology*, 19, 44. doi: 10.1186/s12898-019-0259-3

806