

1 **Colour-assortative mating in a colour-polymorphic lacertid lizard**

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7 Short running title: Colour assortative mating in *Podarcis muralis*

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14 **Abstract**

15 Colour polymorphisms are common in lizards, which provide an excellent model
16 system to study their evolution and adaptive function. The lacertid genus *Podarcis* is
17 particularly interesting because it comprises several polymorphic species. Previous
18 studies with lacertid lizards have tried to explain the maintenance of colour
19 polymorphisms by correlational selection between colour morphs and several
20 phenotypic traits. Particular attention has been paid to their putative role as signals
21 reflecting alternative reproductive strategies under frequency-dependent selection, but
22 the relationship between mating patterns and colour polymorphism has not been
23 previously considered. In this study, we use longitudinal behavioural data obtained
24 during six consecutive breeding seasons (2006-2011) in a free-ranging polymorphic
25 population of *Podarcis muralis* lizards to examine the hypothesis that lizards mate
26 assortatively by colour. We provide spectrophotometric data that confirm the existence
27 of discrete colour morphs and show that morphs are ontogenetically stable once they
28 develop fully in sexually mature individuals. We also present data on the year-to-year
29 variation of relative morph frequencies. Finally, we provide evidence that, over a six-
30 year period, homomorphic male-female pairs in the wild were significantly more
31 common than heteromorphic pairs. Taken together, our results suggest that colour
32 assortative mating may be involved in the maintenance of discrete colour morphs in
33 this and other lacertid species.

34 Keywords: alternative reproductive strategies; assortative mating; colour variation;
35 polymorphism

36 **Introduction**

37 A major challenge in evolutionary biology is to understand the genetic mechanisms
38 and evolutionary processes involved in the maintenance of phenotypic variation,
39 including colour polymorphisms (Roulin 2004; Chunco et al. 2007; Pryke and Griffith
40 2007; McKinnon and Pierotti 2010). Studies with lizards have contributed importantly
41 to this endeavour, and suggest that the lizard model is an ideal one in which to test
42 hypotheses and predictions about the evolution and maintenance of colour
43 polymorphisms (Sinervo and Lively 1996; Zamudio and Sinervo 2000; Vercken et al.
44 2007). Colour polymorphisms are common in lacertids (Arnold and Oveden 2002),
45 and typically consist of lizards exhibiting either white or orange ventral colourations
46 (i.e. throat and/or belly), although some populations feature an additional yellow
47 morph as well as several rare intermediate phenotypes. Polymorphic populations of
48 lizards seem to be particularly abundant in the lacertid genus *Podarcis* (*P.*
49 *melisellensis*, Huyghe et al. 2007, 2009a,b, 2010a,b; *P. muralis*, Cheylan 1988; Sacchi
50 et al. 2007a,b, 2009; Calsbeek et al. 2010; Galeotti et al. 2010; Font et al. 2010; *P.*
51 *gaigeae*, Runemark et al. 2010; *P. vaucheri* and *P. liolepis*, G. Pérez i de Lanuza,
52 unpublished data), which makes it an excellent system in which to study the evolution,
53 maintenance, and adaptive function of genetic colour polymorphisms.

54 While different evolutionary processes may underlie colour polymorphisms
55 (Roulin 2004; Bond 2007; Gray and McKinnon 2007; Roulin and Bize, 2007), work
56 on lizards has mainly focused on the search for phenotypic syndromes associated with
57 discrete colour polymorphisms (e.g., Sinervo and Calsbeek 2006; Calsbeek et al.
58 2010). Several studies have explored potential differences across colour morphs in a
59 number of life-history, behavioural, performance, morphological, ecological and

60 physiological traits. In particular, much attention has been paid to the possibility that
61 lacertid colour polymorphisms reflect the existence of alternative reproductive
62 strategies that would be maintained by frequency-dependent selection (Sinervo and
63 Lively 1996; Sinervo et al. 2007). In *P. melisellensis*, males of different colour morphs
64 have been found to differ in several phenotypic traits (i.e. morphology, fighting
65 ability, corticosterone levels, haemoparasite infection levels, immune response) that
66 hint at the existence of alternative reproductive strategies (Huyghe et al. 2007, 2009a,
67 2010b). In the wall lizard (*P. muralis*) male morphs do not differ in aggressive levels
68 or fighting success, although immune response and susceptibility to stress in captivity
69 appear to covary with colour morphs (Sacchi et al. 2007b, 2009; Galeotti et al. 2010).
70 Calsbeek et al. (2010) recently reported differences among *P. muralis* morphs in
71 haemoparasite infection rate, intensity, and probability of survival. These authors
72 proposed that *P. muralis* morphs experience different multivariate selection pressures,
73 and suggested that such correlational selection may have favoured the evolution of
74 alternative optimal morph-specific phenotypes (Lande and Arnold 1983; Forsman et
75 al. 2008). However, the fact that multiple phenotypic optima may underlie alternative
76 behavioural strategies does not explain why each strategy should be associated with a
77 different ventral colouration (Roulin 2004); indeed, there is no experimental
78 confirmation of the presumed signalling role of ventral colourations or of the
79 information they make available to potential receivers. In conclusion, we are still far
80 from identifying the genetic and evolutionary processes underlying the maintenance of
81 colour polymorphisms in lacertid lizards.

82 Mate choice has been put forward as one of the evolutionary mechanisms that
83 can contribute to maintaining colour polymorphisms (Roulin 2004; Pryke and Griffith
84 2007; Puebla et al. 2007; Reynolds and Fitzpatrick 2007; Elmer et al. 2009). Although

85 the evolution of monotonic directional preferences is predicted for condition-
86 dependent colour traits, in polymorphic systems colour-based mate choice decisions
87 may be morph-specific. This will happen when the reproductive fitness of mating with
88 a given morph is dependent on the morph of both sexes (Roulin 2004; Pryke and
89 Griffith 2009), and will be reflected by the existence of nonrandom assortative or
90 disassortative mating between different colour morphs (Lank 2002; Galeotti et al.
91 2003; Roulin 2004; Gray and McKinnon 2007; Pryke and Griffith 2007; Roulin and
92 Bize 2007).

93 Here we address the possibility that mate choice patterns (i.e. mate
94 recognition/assessment *sensu* Johansson and Jones 2007) may be non-random with
95 respect to colour morph. To this end, we used data collected over six consecutive
96 breeding seasons in a free-ranging polymorphic population of *P. muralis*: 1) to
97 objectively evaluate (i.e. using spectrophotometric methods) the existence of discrete
98 colour morphs, 2) to assess the ontogenetic stability of colour morphs, 3) to study
99 inter-seasonal variation in relative morph frequencies, and 4) to analyse the existence
100 of assortative colour-based mating between different morphs.

101 Materials and methods

102 *Study population*

103 We studied a white-yellow-orange polymorphic population of *P. muralis* from the
104 Cerdanya valley in the south-eastern Pyrenees. In this population, males and females
105 typically exhibit three alternative pure-colour morphs (white, W; yellow, Y; orange,
106 O), although some individuals show intermediate phenotypes consisting of a mosaic of
107 white and orange scales (WO) or a mosaic of yellow and orange scales (YO). Despite

108 extensive sampling, we did not find lizards with an intermediate white and yellow
109 phenotype as described by Calsbeek et al. (2010). Whereas in males the ventral
110 colourations extend over the throat and belly, in females yellow and orange
111 colourations are restricted to the throat (i.e. the belly is always white). All the lizards
112 included in this study came from a homogeneous, continuous population, so our
113 estimates of morph frequency are not confounded by inter-population differences in
114 morph frequencies, which seem common in this species (Cheylan 1988; Sacchi et al.
115 2007a; G. Pérez i de Lanuza, unpublished results).

116 *Colour morph characterization, colour morph development and polymorphism*
117 *stability*

118 Our main study area was a 2.1 Ha continuous patch of abandoned cultivated terraces
119 with artificial stonewalls and sparse vegetation mainly consisting of ashes, rosebushes,
120 hawthorns, and blackthorns. Each spring between 2006 and 2011, we performed field
121 surveys in this area during 4-5 weeks from late May to early July. Overall, we
122 captured 770 animals for which we determined colour morph (eye-based assignment),
123 sex, and age (i.e. juvenile, subadult or adult). We measured body size (as snout-vent
124 length, SVL), body mass, and head width (HW) in all the lizards, and throat
125 colouration in a subsample of adult males and females. To obtain objective
126 measurements of throat colouration we used a USB2000 portable spectrometer with a
127 PX-2 Xenon strobe lamp (Ocean Optics Inc., Dunedin, FL, USA) and standard
128 spectrophotometric techniques (for details see Font et al. 2009; Pérez i de Lanuza and
129 Font 2011). We restricted colour analyses to the 300-700 nm range, which
130 encompasses the visual spectrum of diurnal lizards (Fleishman et al. 1993, 1997;
131 Loew et al. 2002). We measured brightness (Q) and hue (H) according to Endler's

132 Segment Classification method (1990). Additionally, we calculated medium
133 wavelength chroma (MC) as $R_{400-600}/R_{300-700}$, where $R_{400-600}$ and $R_{300-700}$ are
134 the sums of the per cent reflectance between 400 and 600 nm and between 300 and
135 700 nm, respectively. We chose this chroma variable because chromatic differences
136 among colour morphs are due mainly to variation in reflectance between 400 and 600
137 nm (see Figure 1). We also calculated the spectral location of the median reflectance
138 (R_{50} , i.e. the difference between maximum and minimum reflectance divided by two;
139 Marshall et al. 2003).

140 Spectral data were analysed by fitting a two-way ANOVA model with morph
141 and sex as fixed factors. In those cases in which we could not assume data to be
142 normally distributed we used the Scheirer-Ray-Hare test (Sokal and Rohlf 2009).
143 Finally, we used one-way ANOVA or Kruskal-Wallis (i.e. when heteroscedasticity
144 and/or normality could not be safely assumed) tests to look for inter-morph
145 morphometric differences in SVL and body condition. Body condition (BCI) was
146 calculated as the body mass residuals after regressing body mass against SVL (Green
147 2001). We restricted this analysis to adult lizards with fully developed colouration (i.e.
148 $SVL > 56$ mm; see below).

149 Before releasing lizards back at their capture locations, all the individuals were
150 marked by toe-clipping for subsequent identification. We used toe-clipping because
151 this is the most adequate and ethically sound method for durable marking in lizards of
152 this size range (for full details see Perry et al. 2011). Briefly, we clipped toes by
153 cutting their distal two-thirds with a pair of sharp surgical scissors. We clipped a
154 maximum of two toes and always tried to select small digits that did not usually draw
155 blood. Injuries that drew blood were cleaned with alcohol and treated with antibiotic

156 to avoid infection. Lizards that presented natural toe loss were not toe clipped.
157 Repeated annual surveys enabled us to quantify yearly frequencies of each colour
158 morph, while lizard recaptures during consecutive seasons allowed us to assess
159 ontogenetic variation in ventral colouration.

160 *Mating system and assortative pairing*

161 *Podarcis muralis* exhibits a polygynandrous mating system in which adult territorial
162 males patrol and aggressively defend against intruding males an area that typically
163 overlaps the home range of one or more females (Boag 1973; Barbault and Mou 1988;
164 Edsman 1990; Oppliger et al. 2007; our own unpublished observations). As a result,
165 during the breeding season males mate repeatedly with females within their territory,
166 and most copulations occur between members of established pairs that associate in
167 time and space (e.g. basking together, sharing refuges). These observations are
168 consistent with reports of male mate guarding in other lacertid species (e.g. Olsson
169 1993a; Marco and Pérez-Mellado 1999; Martín and López 1999), and suggest that
170 consistent spatiotemporal male-female associations during the breeding season can be
171 used as an indicator of mating pattern.

172 To examine the existence of assortative pairing, we conducted field surveys
173 using two complementary sampling procedures. First, we used data on male-female
174 spatial association obtained from individually identified, marked adult animals during
175 systematic field observations conducted during the first five years at our main study
176 site. For these individuals, spatiotemporal association and interactions (e.g.
177 copulations) among males and females were well documented. Second, in 2010 and
178 2011, we supplemented longitudinal data by sampling pairs of unmarked lizards
179 observed during independent transects in areas adjacent (> 500 m) to our main study

180 site. In this second type of surveys, we used a conservative criterion to record male-
181 female pairs based on previous observations of individually-identified animals: 1) we
182 only recorded pairs in which both lizards could be unambiguously assigned to a
183 morph, 2) pairs involving subadult individuals or young adults were discarded (to
184 avoid the inclusion of lizards without a fully developed colouration; see results), and
185 3) pairs in which lizards were farther than one meter apart were also discarded. To
186 avoid pseudo-replication, transects involving unmarked individuals were conducted
187 only once during the whole sampling period (2010-2011). To test for non-random
188 pairing, we performed a Fisher's exact test on the total pooled dataset. To specifically
189 test for assortative pairing, we used a binomial test comparing the proportion of
190 homomorphic and heteromorphic pairs. Finally, we performed a Spearman correlation
191 analysis with male and female SVL measurements from pairs of marked lizards to test
192 the hypothesis that the pairing system is driven by size-assortative mating.

193 **Results**

194 *Objective colour morph characterization*

195 We obtained reflectance spectra from a total of 249 adult males and 103 females with
196 pure-morph phenotypes (W, Y, O). Intermediate morphs (WO and YO) were
197 discarded for spectrophotometric measurements because the patches of their throat
198 mosaic were often too small to allow consistent measuring (see also Calsbeek et al.
199 2010). Measurement of a subsample of intermediate individuals exhibiting several
200 adjacent scales of the same colour (and thus large enough to allow consistent
201 measurement) confirmed that the colours present in intermediate morphs had the same
202 spectral properties as those of uniformly coloured animals (Figure S1 in sup. mat.).

203 Reflectance spectra from the throats of pure white, yellow and orange adult
204 males and females indicate that colouration does not vary continuously (Figure 1),
205 which supports our eye-based classification into three discrete colour morphs.
206 Differences among ventral colourations are explained by variation in chroma between
207 400 and 600 nm, which gives rise to three discrete spectral steps (see error bars
208 associated with spectra in this range in Figure 1 and distribution of MC among morphs
209 in Figure 2). We found colour morphs to differ in colour variables, but sex and the
210 interaction between morph and sex were not significant (Table 1 summarises Q, MC,
211 H and R_{50} values for the three pure morphs). Finally, we found no differences among
212 morphs in BCI and only males differed slightly in SVL (Table 2). Using post-hoc
213 analyses, we found O males to be larger than W males ($P = 0.026$), but we did not find
214 significant differences between W and Y and O and Y males ($P = 0.44$ and $P = 0.61$,
215 respectively).

216 *Colour morph development and polymorphism stability*

217 Data obtained from lizards recaptured during 2-3 consecutive reproductive seasons
218 (i.e. 28 females and 44 males) indicate that lizards develop their full adult colour by
219 the time they reach a size of 56 mm SVL, which we used as a criterion to exclude
220 individuals without a fully-developed adult colouration. All juveniles sampled were
221 white ventrally. As they grew, some juveniles retained their white colouration as
222 adults, while others developed light yellow pigmentation or a series of isolated orange
223 scales. Subsequently, light-yellow individuals acquired an intense yellow colouration
224 and, in some cases, developed some orange scales (YO intermediate morph). In turn,
225 individuals with orange scales either remained white and orange (WO intermediate
226 morph), changed their white scales to yellow scales (YO) or, more often, developed an

227 overall orange colouration (Figure 3). No individuals examined more than once as
228 adults underwent a colour change, suggesting that colour morphs are stable through
229 adulthood ($N = 30$). At the population level, the polymorphism remained stable over
230 time with only minor fluctuations in the relative frequency of the different morphs
231 (Figure 4).

232 *Assortative pairing*

233 Figure 5 shows the frequency of scored pairs sorted by colour (40 independent pairs of
234 marked lizards and 77 pairs of un-marked lizards). From the subset of individually
235 identified lizards, 26 males were observed in association with females in more than
236 one occasion. Seventeen of these males paired with a single female. In all the
237 remaining cases males were seen repeatedly with the same female and only
238 sporadically with other females. In these cases, we considered the female with which
239 the male paired consistently as his mate. Pairs observed repeatedly were tallied only
240 once.

241 Pairing was not random with respect to male and female colour morphs
242 (Fisher's test, $P < 0.001$), pure-morph homomorphic pairs of lizards being
243 significantly more common than heteromorphic pairs (Binomial test, $N = 94$, $k = 69$, P
244 < 0.001). Due to small sample sizes ($N = 23$), pairings involving intermediate morphs
245 were not included in the analysis (see Table S1 in sup. mat. for raw data). Although
246 SVL measurements were only available for a subset of individually identified lizards,
247 the correlation between male and female SVL within mated pairs was not significant
248 ($N = 17$, $\rho = 0.10$, $P = 0.69$).

249 **Discussion**

250 To our knowledge, no study to date has examined colour-polymorphic assortative
251 mating in *Podarcis* or in other lizards, although a recent study by Huyghe et al.
252 (2010b) found indirect evidence that hinted at the existence of nonrandom mating in
253 populations of *P. melisellensis* with three pure morphs. Here, we provide behavioural
254 evidence of colour-assortative pairing in a wild population of *P. muralis*, which is
255 strongly suggestive of assortative mating in this species. Size-assortative mating
256 cannot explain the pattern reported in this study because we did not find size
257 differences between female morphs (and only a marginal size difference between
258 orange and white-morph males), or a significant correlation between male and female
259 SVL in mated pairs (Olsson 1993b; Cooper and Vitt 1997; Shine et al. 2001).
260 Similarly, spatial data for this population (not shown) reveal that home ranges of
261 lizards from different morphs overlap as often as those of lizards from the same
262 morph. Thus, morphs are not in any way spatially segregated and interact with each
263 other on a daily basis so there are ample opportunities for inter-morph associations
264 (Font et al. 2012; G. Pérez i de Lanuza, E. Font and P. Carazo, unpub. data).

265 The results presented here suggest that mate choice patterns may be involved
266 in the maintenance of colour polymorphisms in *P. muralis* (Roulin and Bize 2007). In
267 polymorphic systems driven by mate choice, disassortative mating is the mechanism
268 that best explains the durability of rare phenotypes (Pryke and Griffith 2007). In
269 contrast, positive assortative mating will normally promote the reduction of gene flow
270 among morphs and, consequently, induce incipient reproductive isolation (Pryke and
271 Griffith 2007; Hughes et al. 2010b). Theoretical arguments, mathematical models and
272 recent empirical evidence all suggest that the processes generating and maintaining
273 colour polymorphism tend to promote speciation (e.g. Gray and McKinnon 2007; Otto
274 et al. 2008; Hugall and Stuart-West 2012). However, the existent of social and

275 ecological constraints on mate availability may curtail the divergence of colour
276 morphs into separate species despite strong assortative mate preferences, in which
277 case assortative mating will contribute to the maintenance of the polymorphism (Pryke
278 2009).

279 Our own results reveal a complex situation in *P. muralis*, with obvious morph-
280 assortative pairing but also a considerable proportion of heteromorphic pairs (38.5%)
281 that could reflect the existence of costs and/or constraints of assortative mating (Pryke
282 2009), or other processes such as condition-dependent variation in mate choice (Bleay
283 and Sinervo 2007). We hence suggest that positive assortative mating could contribute
284 to the maintenance of colour polymorphisms in this species even though it is unlikely
285 that this is the only evolutionary process involved (Pryke 2009). As a matter of fact,
286 colour polymorphisms are widespread in the genus *Podarcis*, which suggest that other
287 selective processes, such as negative frequency dependent selection (Sinervo and
288 Lively 1996), are probably also involved in the maintenance of colour polymorphisms.

289 The findings reported here also contribute to our understanding of the form and
290 development of lacertid colour polymorphisms. First, our data show that there is no
291 sexual dimorphism in the spectral characteristics of male and female morphs; the only
292 observable sexual differences are in the distribution of ventral colorations (i.e. throat
293 and belly in males, only throat in females). Second, spectrophotometric data confirm
294 the existence of discrete colour morphs in both males and females. Available data on
295 the visual system of lacertids (Wagner 1933; Swiezawska 1950; Svoboda 1969;
296 Dücker and Rensch 1973; G. Pérez i de Lanuza and E. Font, in preparation) and other
297 diurnal lizards (Fleishman et al. 1997, 2011; Loew et al. 2002; Bowmaker et al. 2005)
298 suggest that *Podarcis* lizards most likely perceive these colour morphs as discrete

299 phenotypes. Therefore, and given that morph differences are explained by variation
300 within the human visible range (i.e. 400-700 nm), an eye-based classification seems to
301 be an adequate procedure for discriminating colour morphs in this species (for a
302 discussion of the pros and cons of eye-based classifications of ventral colourations in
303 lacertids see Vercken et al. 2007, 2008; Cote et al. 2008). Our results also show that
304 colour morphs in adult *P. muralis* appear to be ontogenetically stable. All
305 developmental changes observed in throat and belly colouration affected subadult
306 and/or young adult lizards (i.e., SVL < 56 mm), so criteria used to identify adult,
307 stable morphs should be taken into account in comparisons among morphs. For
308 example, Sacchi et al. (2007b, 2009) classified individuals with SVL larger than 50
309 mm as adults, which could have led to overestimating the proportion of white morph
310 lizards in Italian populations of *P. muralis* (Sacchi et al. 2007a; see also Calsbeek et
311 al. 2010).

312 A host of interesting questions remain to be examined in more detail by future
313 studies. In particular, it is often assumed that colour polymorphisms in lacertids are, as
314 in other polymorphic lizards, genetically determined (Sacchi et al. 2007a,b; Calsbeek
315 et al. 2010; Runemark et al. 2010), but controlled matings have not been conducted to
316 confirm the heritability of colour morphs. Therefore, a crucial goal of future research
317 should be to decipher the genetic basis underlying colour polymorphism in this and in
318 other polymorphic species of lacertid lizards.

319

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328 **References**

- 329 Arnold EN, Oveden D. 2002. A Field Guide to the Reptiles and Amphibians of Britain
330 and Europe, 2nd edn. London, UK: Collins.
- 331 Barbault R, Mou YP. 1988. Population dynamics of the common lizard, *Podarcis*
332 *muralis*, in southwestern France. *Herpetologica*. 44:38-47.
- 333 Bleay C, Sinervo B. 2007. Discrete genetic variation in mate choice and a condition-
334 dependent preference function in the sideblotched lizard: implications for the
335 formation and maintenance of coadapted gene complexes. *Behav Ecol*. 18:304-310.
- 336 Boag DA. 1973. Spatial relationships among members of a population of wall lizards.
337 *Oecologia*. 12:1-13.
- 338 Bond AB. 2007. The Evolution of color polymorphism: crypticity, searching images,
339 and apostatic selection. *Annu Rev Ecol Evol Syst*. 38:489-514.
- 340 Bowmaker JK, Loew ER, Ott M. 2005. The cone photoreceptors and visual pigments
341 of chameleons. *J Comp Physiol A*. 191:925-932.

342 Calsbeek B, Hasselquist D, Clobert J. 2010. Multivariate phenotypes and the potential
343 for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour
344 morphs. J Evol Biol. 23:1138-1147.

345 Cheylan M. 1988. Variabilité phénotypique du Lézard des murailles *Podarcis muralis*
346 sur les îles de la côte provençale. France Rev Ecol Terre-Vie. 43:287-321.

347 Chunco AJ, McKinnon JS, Servedio MR. 2007. Microhabitat variation and sexual
348 selection can maintain male color polymorphisms. Evolution. 61:2504-2515.

349 Cooper WE, Vitt LJ. 1997. Maximizing male reproductive success in the broad-
350 headed skink (*Eumeces laticeps*): preliminary evidence for mate guarding, size-
351 assortative pairing, and opportunistic extra-pair mating. Amphibia-Reptilia. 18:59-73.

352 Cote J, Le Galliard JF, Rossi JM, Fitze PS. 2008. Environmentally induced changes in
353 carotenoid-based coloration of female lizards: a comment on Vercken et al. J Evol
354 Biol. 21:1165-1172.

355 Dücker GV, Rensch B. 1973. Die visuelle Lernkapazität von *Lacerta viridis* und
356 *Agama agama*. Z Tierpsychol. 32:209-214.

357 Edsman L. 1990. Territoriality and competition in wall lizards [PhD Thesis].
358 Stockholm: Univ Stockholm.

359 Elmer KR, Lehtonen TK, Meyer A. 2009. Color assortative mating contributes to
360 sympatric divergence of neotropical cichlid fish. Evolution. 63:2750-2757.

361 Endler JA. 1990. On the measurement and classification of colour in studies of animal
362 colour patterns. Biol J Linn Soc. 41:315-352.

363 Fleishman LJ, Loew ER, Leal M. 1993. Ultraviolet vision in lizards. *Nature*. 365:397.

364 Fleishman LJ, Bowman M, Saunders D, Miller WE, Rury MJ, Loew ER. 1997. The
365 visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. *J*
366 *Comp Physiol A*. 181:446-460.

367 Fleishman LJ, Loew ER, Whiting MJ. 2011. High sensitivity to short wavelengths in a
368 lizard and implications for understanding the evolution of visual systems in lizards.
369 *Proc R Soc Lond B*. 278: 2891-2899.

370 Font E, Pérez i de Lanuza G, Sampedro C. 2009. Ultraviolet reflectance and cryptic
371 sexual dichromatism in the ocellated lizard, *Lacerta (Timon) lepida* (Squamata:
372 Lacertidae). *Biol J Linn Soc*. 97:766-780.

373 Font E, Carazo P, Pérez i de Lanuza G, Barbosa D. 2010. Comportamiento y
374 comunicación animal: ¿Qué nos enseñan los lagartos? *Acta Zool Lilloana*. 54:12-35.

375 Font E, Barbosa D, Sampedro C, Carazo P. 2012. Social behaviour, chemical
376 communication, and adult neurogenesis: Studies of scent mark function in *Podarcis*
377 wall lizards. *Gen Comp Endocr*. 177:9-17.

378 Forsman A, Ahnesjö J, Caesar S, Karlsson M. 2008. A model of ecological and
379 evolutionary consequences of color polymorphism. *Ecology*. 89:34-40.

380 Galeotti P, Rubolini D, Dunn PO, Fasola M. 2003. Colour polymorphism in birds:
381 causes and functions. *J Evol Biol*. 16:635-646.

382 Galeotti P, Pellitteri-Rosa D, Sacchi R, Gentili A, Pupin F, Rubolini D, Fasola M.
383 2010. Sex-, morph- and size-specific susceptibility to stress measured by

384 haematological variables in captive common wall lizard *Podarcis muralis*. Comp
385 Biochem Physiol A. 157:354-363.

386 Gray SM, McKinnon JS. 2007. Linking color polymorphism maintenance and
387 speciation. Proc R Soc B. 22:71-79.

388 Green AJ. 2001. Mass/length residuals: measures of body condition or generators of
389 spurious results? Ecology. 82:1473-1483.

390 Hugall AF, Stuart-Fox D. 2012. Accelerated speciation in colour-polymorphic birds.
391 Nature. 485:31-34.

392 Huyghe K, Vanhooydonck B, Herrel A, Tadić Z, Van Damme R. 2007. Morphology,
393 performance, behavior and ecology of the lizard *Podarcis melisellensis*. Integr Comp
394 Biol. 47:211-220.

395 Huyghe K, Husak JF, Herrel A, Tadić Z, Moore IT, Van Damme R, Vanhooydonck B.
396 2009a. Relationships between hormones, physiological performance and
397 immunocompetence in a color-polymorphic lizard species, *Podarcis melisellensis*.
398 Horm Behav. 55:488-494.

399 Huyghe K, Herrel A, Adriaens D, Tadić Z, Van Damme R. 2009b. It is all in the head:
400 morphological basis for differences in bite force among colour morphs of the
401 Dalmatian wall lizard. Biol J Linn Soc. 96:13-22.

402 Huyghe K, Van Oystaeyen A, Pasmans F, Tadić Z, Vanhooydonck B, Van Damme R.
403 2010a. Seasonal changes in parasite load and a cellular immune response in a colour
404 polymorphic lizard. Oecologia. 163:867-874.

405 Huyghe K, Small M, Vanhooydonck B, Herrel A, Tadić Z, Van Damme R, Backeljau
406 T. 2010b. Genetic divergence among sympatric colour morphs of the Dalmatian wall
407 lizard (*Podarcis melisellensis*). *Genetica*. 138:387-393.

408 Johansson BJ, Jones TM. 2007. The role of chemical communication in mate choice.
409 *Biol Rev*. 82:265-289.

410 Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters.
411 *Evolution*. 37:1210-1226.

412 Lank DB. 2002. Diverse processes maintain plumage polymorphisms in birds. *J Avian*
413 *Biol*. 33:327-330.

414 Loew ER, Fleishman LJ, Foster RG, Provencio I. 2002. Visual pigments and oil
415 droplets in diurnal lizards: a comparative study of Caribbean anoles. *J Exp Biol*.
416 205:927-938.

417 Marco A, Perez-Mellado V. 1999. Mate guarding, intrasexual competition and mating
418 success in males of the non-territorial lizard *Lacerta schreiberi*. *Ethol Ecol Evol*.
419 11:279-286.

420 Marshall NJ, Jennings K, McFarland WN, Loew ER, Losey GS. 2003. Visual biology
421 of Hawaiian coral reef fishes. II. Colors of Hawaiian coral reef fish. *Copeia*. 2003:455-
422 466.

423 Martín J, López P. 1999. Nuptial coloration and mate guarding affect escape decision
424 of male lizards *Psammodromus algirus*. *Ethology*. 105:439-447.

425 McKinnon JS, Pierotti MER. 2010. Colour polymorphism and correlated characters:
426 genetic mechanisms and evolution. *Mol Ecol.* 19:5101-5125.

427 Olsson M. 1993a. Contest success and mate guarding in male sand lizards, *Lacerta*
428 *agilis*. *Anim Behav.* 46:408-409.

429 Olsson M. 1993b. Male preference for large females and assortative mating for body
430 size in the sand lizard (*Lacerta agilis*). *Behav Ecol Sociobiol.* 32:337-341.

431 Oppliger A, Degen L, Bouteillier-Reuter C, John-Alder HB. 2007. Promiscuity and
432 high level of multiple paternity in common wall lizards (*Podarcis muralis*): data from
433 microsatellite markers. *Amphibia-Reptilia.* 28:301-3303.

434 Otto SP, Servedio MR, Nuismer SL. 2008. Frequency-dependent selection and the
435 evolution of assortative mating. *Genetics.* 179:2091-2112.

436 Pérez i de Lanuza G, Font E. 2011. Lizard blues: blue body colouration and ultraviolet
437 polychromatism in lacertids. *Rev Esp Herp.* 24:67-84.

438 Perry G, Wallace MC, Perry D, Curzer H, Muhlberger P. 2011. Toe clipping of
439 amphibians and reptiles: science, ethics, and the law. *J Herpetol.* 45:547-555.

440 Pryke SR. 2009. Sex chromosome linkage of mate preferences and color signal
441 maintains assortative mating between interbreeding finch morphs. *Evolution.* 64:1301-
442 1310.

443 Pryke SR, Griffith SC. 2007. The relative role of male versus female mate choice in
444 maintaining assortative pairing among discrete colour morphs. *J Evol Biol.* 20:1512-
445 1521.

446 Pryke SR, Griffith SC. 2009. Genetic incompatibility drives sex allocation and
447 maternal investment in a polymorphic finch. *Science*. 323:1605-1607.

448 Puebla O, Bermingham E, Guichard F, Whiteman E. 2007. Colour pattern as a single
449 trait driving speciation in *Hypoplectrus* coral reef fishes? *Proc R Soc Lond B*.
450 274:1265-1271.

451 Reynolds RG, Fitzpatrick BM. 2007. Assortative mating in poison-dart frogs based on
452 an ecologically important trait. *Evolution*. 61:2253-2259.

453 Roulin A. 2004. The evolution, maintenance and adaptive function of genetic colour
454 polymorphism in birds. *Biol Rev*. 79:815-848.

455 Roulin A, Bize P. 2007. Sexual selection in genetic colour-polymorphic species: a
456 review of experimental studies and perspectives. *J Ethol*. 25:99-105.

457 Runemark A, Hansson B, Pafilis P, Valakos ED, Svensson EI. 2010. Island biology
458 and morphological divergence of the Skyros wall lizard *Podarcis gaigeae*: a combined
459 role for local selection and genetic drift on color morph frequency divergence? *BMC*
460 *Evol Biol*. 10:269.

461 Sacchi R, Scali S, Pupin F, Gentili A, Galeotti P, Fasola M. 2007a. Microgeographic
462 variation of colour morph frequency and biometry of common wall lizards. *J Zool*.
463 273:389-396.

464 Sacchi R, Rubolini D, Gentili A, Pupin F, Razzetti E, Scali S, Galeotti P, Fasola M.
465 2007b. Morph-specific immunity in males of the common wall lizard, *Podarcis*
466 *muralis*. *Amphibia-Reptilia*. 28:408-412.

467 Sacchi R, Pupin F, Gentili A, Rubolini D, Scali S, Fasola M, Galeotti P. 2009. Male-
468 male combats in a polymorphic lizard: residency and size, but not color, affect fighting
469 rules and contest outcome. *Aggr Behav.* 35:274-283.

470 Shine R, O'Connor D, Lemaster MP, Mason RT. 2001. Pick on someone your own
471 size: ontogenetic shifts in mate choice by male garter snakes result in size assortative
472 mating. *Anim Behav.* 61:1133-1141.

473 Sinervo B, Calsbeek R. 2006. The developmental, physiological, neural, and genetical
474 causes and consequences of frequency-dependent selection in the wild. *Annu Rev*
475 *Ecol Evol Syst.* 37:581-610.

476 Sinervo B, Lively CM. 1996. The rock-paper-scissors game and the evolution of
477 alternative male reproductive strategies. *Nature.* 380:240-243.

478 Sinervo B, Heulin B, Surget-Groba Y, Clobert J, Miles DB, Corl A, Chaine A, Davis
479 A. 2007. Models of density-dependent genic selection and a new rock-paper-scissors
480 social system. *Am Nat.* 170:663-680.

481 Sokal RR, Rohlf FS. 2009. *Biometry*, 2nd edn. New York, USA: Freeman Co.

482 Svoboda B. 1969. Die Bedeutung von Farb-, Form- und Geruchsmerkmalen für das
483 Vermeidenlernen von Beuteobjekten bei *Lacerta agilis* L [dissertation]. Wien:
484 Universität Wien.

485 Swiezawska K. 1950. Colour-discrimination of the sand lizard, *Lacerta agilis* L. *Bull*
486 *Intern Acad Polish Sci Lett Ser B.* 569:1-20.

- 487 Vercken E, Massot M, Sinervo B, Clobert J. 2007. Colour variation and alternative
488 reproductive strategies in females of the common lizard *Lacerta vivipara*. J Evol Biol.
489 20:221-232.
- 490 Vercken E, Sinervo B, Clobert J. 2008. Colour variation in female common lizards:
491 why we should speak of morphs, a reply to Cote et al. J Evol Biol. 21:1160-1164.
- 492 Wagner H. 1933. Über den Farbensinn der Eidechsen. Z Vergl Physiol. 18:378-392.
- 493 Zamudio K, Sinervo B. 2000. Polygyny, mate-guarding, and posthumous fertilization
494 as alternative male mating strategies. Proc Natl Acad Sci USA. 97:1427-1432.

495 **Figure 1.** Throat reflectance spectra from pure *P. muralis* adult male (a) and female
496 (b) morphs. W, Y and O represent white, yellow and orange morphs respectively.
497 Spectra from white, yellow and orange scales from intermediate white-orange and
498 yellow-orange morphs are similar to those depicted here (see Figure S1 in sup. mat.).
499 Vertical lines: error bars (± 1 SEM).

500 **Figure 2.** Distribution of medium wavelength chroma (MC) measurements (males and
501 females considered together) from each pure morph. W, Y and O represent white,
502 yellow and orange morphs respectively.

503 **Figure 3.** Developmental trajectories of colour morphs in *P. muralis* reconstructed
504 from recapture data. Arrows indicate direction of possible changes in colouration over
505 time. Black arrows represent most frequent transitions and numbers over the arrows
506 indicate the number of observed individuals for each transition in the population (f =
507 females; m = males). Counts in transitions from juveniles to subadults/young adults
508 consider one year old animals with SVL < 56 mm (which always develop their ventral
509 colouration from white juveniles). Transitions from subadults/young adults to adults
510 were calculated considering only individuals captured for the first time with SVL < 56
511 mm, and recaptured during the following spring as adults.

512 **Figure 4.** Changes in colour morph frequencies during a six-year period. Sample sizes
513 for each sex and year are provided in brackets. *N* represents the total sample size
514 across the six years.

515 **Figure 5.** Number of male-female pairs classified by colour morph from a total of 117
516 pairs.

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521 **Table 1.** Descriptive statistics and results of statistical analyses for colour variables:
 522 brightness (Q), medium wavelength chroma (MC), hue (H, in degrees) and spectral
 523 location of middle point (R_{50} , in nm) for each morph and sex. W, Y and O represent
 524 the white, yellow and orange morphs respectively. Asterisks indicate statistically
 525 significant results ($P < 0.001$ in all cases). See sample sizes in Figure 1.

		Q	MC	H	R₅₀
males	W	38926 ± 650	0.52 ± 0.00	49.50 ± 0.65	416.54 ± 2.50
	Y	33500 ± 726	0.46 ± 0.00	37.83 ± 0.60	496.10 ± 3.17
	O	30790 ± 1079	0.40 ± 0.01	25.56 ± 0.82	532.39 ± 5.40
females	W	41174 ± 875	0.50 ± 0.00	46.38 ± 0.01	427.07 ± 4.55
	Y	38955 ± 1380	0.47 ± 0.00	39.06 ± 0.74	488.54 ± 5.32
	O	30237 ± 1546	0.40 ± 0.01	26.00 ± 1.15	536.29 ± 6.58
	morph	$H = 14.70^*$	$H = 39.82^*$	$H = 38.6^*$	$F_{1,346} = 329^*$
	sex	$H = 0.01$	$H = 0.01$	$H = 0.02$	$F_{1,346} = 0.57$
	interaction	$H = 1.43$	$H = 0.66$	$H = 0.77$	$F_{1,346} = 0.12$

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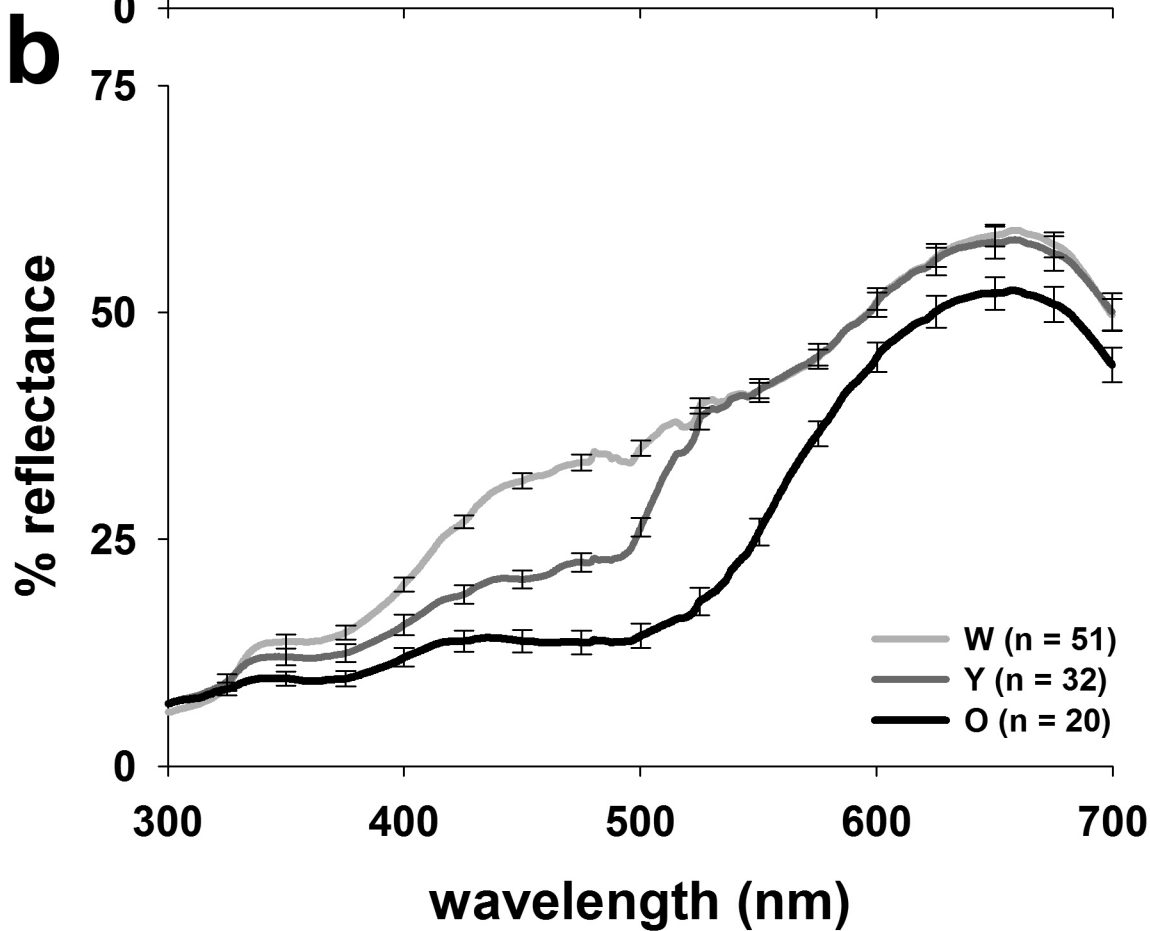
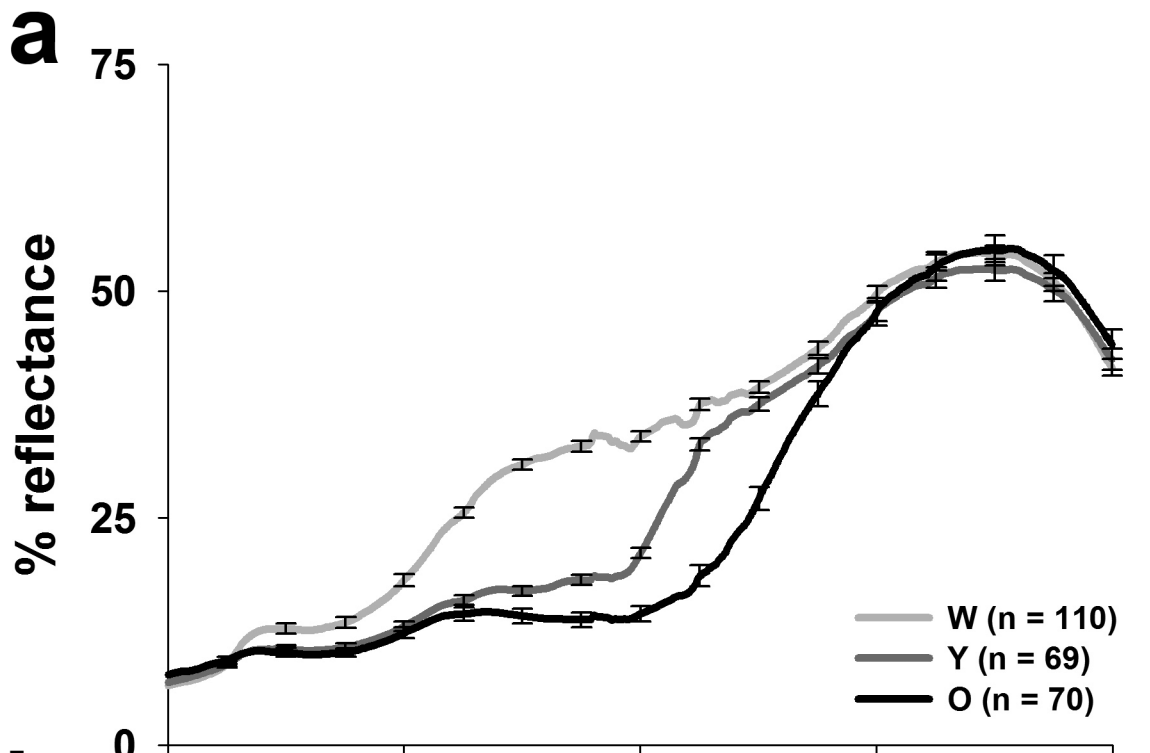
527 **Table 2.** Mean values of morphometric variables SVL (i.e. snout to vent length) and
 528 BCI (i.e. body condition index), and results of comparisons among morphs. Values
 529 were log-transformed prior to the analyses. W, Y and O represent the white, yellow
 530 and orange morphs respectively.

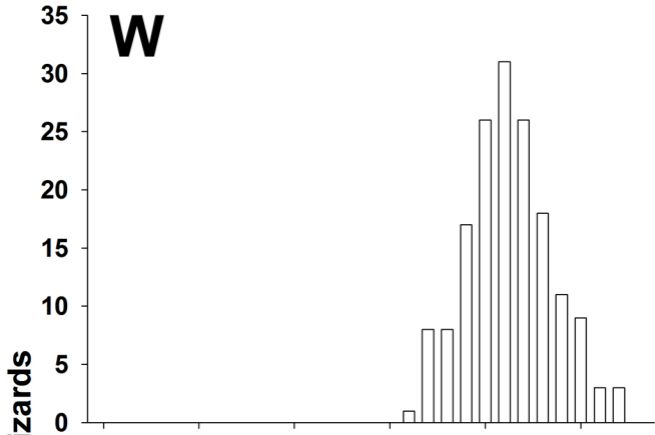
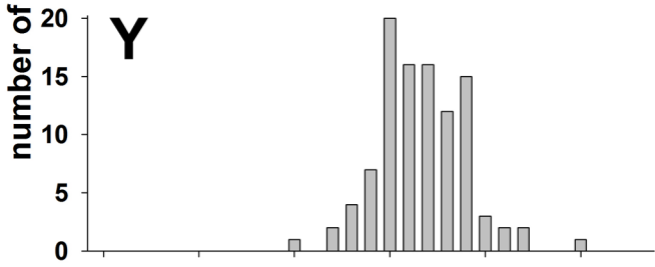
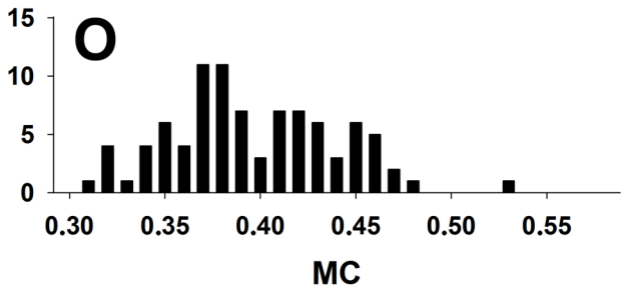
	SVL (mm)		BCI	
	females	males	females	males
W	61.78 ± 0.73	62.57 ± 0.51	-0.16 ± 0.10	0.21 ± 0.06
Y	63.56 ± 0.77	63.63 ± 0.51	-0.28 ± 0.15	0.18 ± 0.08
O	63.38 ± 0.75	64.77 ± 0.57	-0.49 ± 0.17	0.02 ± 0.11
	$F_{2,65} = 1.71$	$F_{2,168} = 3.58$	$F_{2,65} = 1.071$	$\chi^2 = 2.58$
	$P = 0.19$	$P = 0.03$	$P = 0.35$	$P = 0.28$

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533



W**Y****O****MC**

juveniles

subadults/
young adults

adults



$f=56/m=63$

$f=25/m=25$

$f=29/m=35$



$f=8/m=11$

$f=2/m=1$

$f=1/m=1$

$f=4/m=6$

$f=1/m=0$

$f=0/m=2$

$f=8/m=9$



