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1 **Male ultraviolet reflectance and female mating history influence female**
2 **mate choice and male mating success in a polyandrous lizard**

3

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19

20 **Short title:** UV reflectance influences female mate choice

21 **Abstract**

22 Pre-copulatory female mate choice based on male ultraviolet (UV) coloration has been
23 demonstrated in several vertebrate species, but post-copulatory mechanisms have been largely
24 overlooked. Here, we investigated female mate preference based on male UV coloration in the
25 common lizard *Zootoca vivipara*, in which males display conspicuous UV coloration on their
26 throat. During two successive years, we staged sequential mating trials between females and
27 four different males with UV-reduced or control belly and throat coloration. We recorded pre-
28 copulatory female behavior, copulation behavior and assigned paternity to all offspring.
29 Females were more aggressive towards UV-reduced males and, in one year, UV-reduced
30 males had a lower probability of siring at least one egg (fertilization success) during the last
31 mating trials. However, in one year, copulation was shorter with control males. Altogether,
32 our results suggest that females exert subtle pre-copulatory mate preference based on male
33 UV ornaments and, conditional on the study year and female mating history, some degree of
34 post-copulatory preference for UV-control males leading to differential male fertilization
35 success. This study suggests that UV-based female mate choice may be more widespread than
36 previously thought vertebrates, and emphasize the importance to use a study design well
37 adapted to the species reproductive behavior.

38

39 **Key-words:** Color signals – Female choice – Fertilization – Paternity – Post-copulatory
40 selection – Sexual selection – Ultraviolet – *Zootoca vivipara*

41

42 **Introduction**

43 Female mate choice is a major component of sexual selection that drives the evolution of
44 male ornaments (Andersson, 1994). Choosing high quality males may increase female
45 reproductive success (Andersson, 1994; Kokko *et al.*, 2003) by providing females with
46 resources increasing their survival or fecundity (direct benefits; e.g. access to good territory,
47 paternal care, protection against predators) or with alleles enhancing the viability and/or
48 attractiveness of their offspring (indirect benefits; "good genes" and "sexy sons", Kirkpatrick
49 & Ryan, 1991; Andersson, 1994; Johnstone, 1995). Females can assess males using signals
50 that correlate consistently with male quality, and ultimately with those direct and indirect
51 benefits (e.g. Cooper & Vitt, 1993; Welch, Semlitsch, & Gerhardt, 1998; Darragh *et al.*,
52 2017). In particular, many animal species exhibit colorful ornaments that convey an honest
53 information about male age, phenotypic condition, or genotypic quality (Senar, 2006;
54 Bradbury & Vehrencamp, 2011; Weaver, Koch, & Hill, 2017).

55 Color signals can be produced by the deposition of integumentary pigments (e.g.
56 melanin and carotenoids), by coherent light-scattering nanostructures (i.e. structural
57 coloration), or by a combination of both (Grether, Kolluru & Nersissian, 2004; Shawkey &
58 D'Alba, 2017; Fan *et al.*, 2019). While the role of pigment-based colors in sexual selection
59 has received much scientific attention (Svensson & Wong, 2011; Roulin, 2016), a growing
60 body of work has emerged in the past two decades showing that structural colors, such as
61 ultraviolet, could also function as sexual signals (Prum 2006; Kemp *et al.* 2012, 2015). Many
62 vertebrate species display structural coloration that reflects light in the ultraviolet (UV) range
63 (e.g. Andersson, Örnborg, & Andersson, 1998; Siebeck, 2004; Ries *et al.*, 2008; Badiane,
64 Carazo, & Font, 2018) and have a visual system sensitive to UV light (Bowmaker, 2008;
65 Cronin & Bok, 2016). We have now good evidence that UV coloration can be sexually
66 dichromatic (Hunt *et al.*, 1998; Names *et al.*, 2019) and act as honest, condition-dependent

67 indicator of male quality (e.g. Keyser & Hill, 1999, 2000; Griggio, Zanollo, & Hoi, 2010;
68 Pérez i de Lanuza, Carazo & Font, 2014). Female mate choice based on male UV coloration
69 has been demonstrated in birds (e.g. Hunt *et al.*, 1999), fishes (e.g. Kodric-Brown & Johnson,
70 2002), amphibians (e.g. Secondi, Lepetz, & Théry, 2012) and lizards (e.g. Bajer *et al.*, 2010).
71 Most studies investigating the effect of UV coloration on female mate choice focused on pre-
72 copulatory mechanisms while post-copulatory mechanisms remain rarely tested. Only
73 Johnsen *et al.* (1998) investigated these aspects and found that the UV coloration of male
74 bluethroats (*Luscinia s. svecica*) positively influenced social and genetic mate choice.

75 Many lizard species display ultraviolet color patches that often evolve under sexual
76 selection (e.g. Thorpe & Richard, 2001; Font & Molina-Borja, 2004; Martin *et al.*, 2013;
77 MacGregor *et al.*, 2017). UV coloration in lizards seems to function as honest indicator of
78 male quality (e.g. Whiting *et al.*, 2006; Molnár *et al.*, 2012; Pérez i de Lanuza, Carazo, &
79 Font, 2014) and has been shown to influence social aggressiveness, dominance, and contest
80 outcome during male-male competition (Stapley & Whiting, 2006; Bajer *et al.*, 2011; Martin
81 *et al.*, 2016; Names *et al.*, 2019). For example, in European green lizards *Lacerta viridis*, UV
82 coloration signals male quality (Molnár *et al.*, 2012, 2013), determines male fighting success
83 (Bajer *et al.*, 2011), and predicts female mate choice (Bajer *et al.*, 2010). Furthermore, female
84 mate choice based on male UV coloration has been shown in only two other lizard species
85 (Bajer *et al.*, 2010; Lisboa *et al.*, 2017) and suggested in one other (Olsson *et al.*, 2010).
86 However, none of these studies tested the influence of UV signaling on male mating success.

87 Here, we investigated whether male UV coloration influences behavioral mate
88 preferences of females, mating behavior and male mating success in the common lizard
89 *Zootoca vivipara*. Common lizards occupy overlapping home ranges (Massot *et al.*, 1992) and
90 have a promiscuous mating system characterized by multiple mating in both sexes (Laloi *et*
91 *al.*, 2004). Male common lizards exhibit a whitish coloration on their throat that strongly

92 reflects UV light (Martin *et al.*, 2013; Bonnaffé *et al.*, 2018). Mating is under partial male
93 control in common lizards (Fitze *et al.*, 2005; Fitze & Le Galliard, 2008), but females can also
94 select males by resisting mating and by sperm selection with multiple mating (Laloi *et al.*,
95 2004, 2011; Fitze *et al.*, 2005; Fitze, Cote, & Clobert, 2010). During two successive years, we
96 presented females sequentially with four different males with either a control or a reduced UV
97 reflectance on their throat and belly, while controlling for other traits important for female
98 mate choice. We quantified female resistance behavior as well as pairing success and
99 copulation duration to gain insights into pre-copulatory mechanisms of choice. To investigate
100 post-copulatory mechanisms and quantify male mating success, we performed paternity
101 analyses to assign offspring to males from both UV treatments. This study design allows us to
102 test two main hypotheses. First, we hypothesize that females use male UV coloration to reject
103 or accept a mating event with a male. If so, we expect females to resist more (biting more and
104 flipping their body more often to escape) mating attempts initiated by UV-reduced males
105 compared to UV-control males. Pairing success and copulation duration should also be higher
106 for UV-control than for UV-reduced males. Second, we expect that, if cryptic female choice
107 occurs, fertilization and reproductive success should be higher for UV-control males.

108 **Materials and methods**

109 *Study species*

110 The common lizard, *Zootoca vivipara*, is a small lacertid (45-70 mm) distributed across
111 Eurasia. In our study site, animals reach sexual maturity at one or two years of age and mating
112 takes place in May (Fitze *et al.*, 2005). Females are ovoviviparous and, after 2-3 months of
113 gestation, give birth to 1-12 eggs depending on female age and body size (Massot *et al.*,
114 1992). Adult males have a whitish throat and a conspicuous belly ranging from yellow to dark
115 red, interspersed with numerous black spots. Females display a duller ventral coloration
116 ranging from cream to orange with fewer black spots than males (Bauwens, 1987; Cote *et al.*,

117 2008). In addition, the ventral coloration shows a secondary reflectance peak in the UV range,
 118 which is especially pronounced on males' throat (Martin *et al.*, 2013). UV chroma of the
 119 throat and belly coloration increases with age and size in males (Bonnaffé *et al.*, 2018).

120 *Sampling and morphometric measurements*

121 In 2012 and 2013, we captured by hand 183 adult males (85 in 2012 and 99 in 2013, 51-62
 122 mm) and 52 adult females (24 in 2012 and 28 in 2013, 57-71 mm) at the Centre de Recherche
 123 en Ecologie Expérimentale et Prédictive (CEREEP-Ecotron IleDeFrance, 48°17'N, 2°41'E),
 124 where males and females were maintained in separate 100-m² enclosures since 2011. Males
 125 were captured before their last molt at the onset of their sexual activity. Females were
 126 captured 10-15 days later once they emerged from wintering. Captures occurred in mid-March
 127 2012 but early April 2013 because of annual differences in weather conditions and phenology.

128 We brought the lizards to the laboratory and measured their snout-vent length (SVL; ± 1
 129 mm) and body mass (± 1 mg). We found no differences in female SVL (ANOVA, $F_{1,50} = 2.00$,
 130 $p = 0.16$) and body mass ($F_{1,50} = 3.14$, $p = 0.08$), nor in male body mass ($F_{1,181} = 0.38$, $p =$
 131 0.54) between the two study years, but males were larger in 2012 than in 2013 (SVL: $F_{1,181} =$
 132 5.25 , $p = 0.02$, $\beta = 0.72 \pm 0.32$ mm). We also obtained reflectance spectra of the throat and
 133 belly (2-3 measures per location) of each male using a spectrophotometer (see Martin *et al.*
 134 2013 for material details). We then calculated brightness (total reflectance), yellow-red hue
 135 (wavelength of maximal reflectance), yellow-red saturation (difference between maximal
 136 reflectance over the range 450-700 nm and reflectance value at 450 nm divided by average
 137 reflectance over the range 300-700 nm), throat UV hue (wavelength of maximal reflectance
 138 between 300 nm and 400 nm) and throat UV chroma (proportion of the UV reflectance
 139 relative to the total reflectance, see Martin *et al.*, 2013 for more details). The throat and
 140 ventral parts have different colors in this species (UV-white throats and yellow-red bellies), so
 141 we used the most adequate color variable to characterize them. Males displayed higher throat

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142 UV hue (ANOVA, $F_{1,181} = 21.83$, $p < 0.0001$, $\beta = 3.29 \pm 0.70$), and lower yellow-red hue and
143 yellow-red saturation in 2013 than in 2012 (yellow-red hue: $F_{1,181} = 7.27$, $p = 0.008$, $\beta = -5.94$
144 ± 2.2 ; yellow-red saturation: $F_{1,181} = 21.83$, $p < 0.001$, $\beta = -0.08 \pm 0.03$) but had similar
145 brightness ($F_{1,181} = 1.41$, $p = 0.24$) and throat UV chroma ($F_{1,181} = 1.14$, $p = 0.29$) between
146 years.

147 We also quantified male head morphology using a digital caliper; we measured head
148 length (from tip of the nose to the head skull-vertebral column articulation), head height
149 (maximum height at the highest part posterior to orbita), head width (width at the maximum
150 lateral extent), quadrate length, and coronoid length to the nearest 0.01 mm in all but one
151 male. All measurements were highly correlated within the same individual (Spearman
152 correlation, $r > 0.33$, all $p < 0.001$) and most traits showed yearly variation in their mean
153 similar to SVL. We therefore extracted a single metric of head size by a centered and scaled
154 principal component analysis using the *dudi.pca* procedure in *Ade4* package (Chessel, Dufour,
155 & Thioulouse, 2004). The first dominant axis (PC1) explained more than 62% of the inter-
156 individual variation in head measurements and thus could be used as a head size metric.
157 Individual scores for PC1 were positively correlated with body size ($r = 0.65$, $p < 0.0001$) but
158 not with throat UV chroma ($r = -.04$, $p = 0.54$). Males had smaller head size in 2013 than in
159 2012 ($F_{1, 181} = 6.46$, $p = 0.01$, $\beta = -0.66 \pm 0.26$).

160 Females were housed in large plastic boxes (45×29×22 cm), in which all behavioral
161 tests took place after 5-6 days of acclimation to minimize stress. Males were housed in
162 smaller plastic boxes (18×12×12 cm) and transferred to the female's terrarium prior to each
163 behavioral test. All terraria were layered with sand, equipped with a small water dish, two
164 hides and a black PVC plate used for basking (4×9 cm). An incandescent bulb (25 W) and
165 white light UV-B neon tubes (Reptisun 10.0 UVB, Zoomed) provided heat and light for 8

166 hours a day. Food (crickets, *Acheta domesticus*) and water were provided *ad libitum* during
167 the experiment.

168 *Color manipulation*

169 To manipulate temporarily male UV reflectance within the natural range of variation (Martin
170 *et al.*, 2015, 2016; Names *et al.* 2019), we used odorless UV-blocking (290-400 nm) inorganic
171 agents (zinc oxide and titan dioxide) mixed with a fat combination of petroleum jelly and
172 liquid paraffin (respectively, 6:4:50:40 for 100 g). Males of the control group were treated
173 with the fat combination and males of the UV-reduced treatment with the fat combination
174 mixed with the inorganic agents. The combination was applied on the males' ventral skin with
175 a soft paintbrush from the tip of the nose to the anal plate. To validate our protocol, we
176 measured the gular reflectance of randomly selected male lizards ($N = 7$ per group) before and
177 after application of fat (control group) or of the UV-reducing treatment (UV-reduced group).
178 Half an hour after the application, this treatment reduced UV reflectance within the natural
179 range of variation of UV chroma (see Appendix 1), and although the effect faded with time, it
180 persisted for at least two hours after application.

181 *Mate choice trials*

182 We designed sequential mating trials by pooling males into 52 quartets (24 in 2012 and 28 in
183 2013). This design mimicked the reproductive behavior of common lizards, as highly mobile
184 males likely approach resident females in a sequential manner during the mating season.
185 Males of the same quartet were matched by SVL (± 2 mm), body mass (± 600 mg) and gular
186 as well as ventral coloration. For each quartet, two lizards were randomly attributed to the
187 control group and to the UV-reduced group. We found no differences in morphology and
188 coloration between UV-control and UV-reduced individuals prior to the experiment
189 (Student's t-tests, all $p > 0.27$). Each male quartet was assigned to a single female according
190 to their rank for SVL, such that larger females could mate with larger males (SVL difference

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191 between males and females, mean = 6.85 mm, range = 3-11). This procedure avoided size
192 mismatches so that we could focus on the role of UV coloration in mate choice, given the
193 significant assortative mating by size in the common lizard (Richard *et al.*, 2005).

194 Each female encountered each of the four males in a random sequence of male UV
195 treatments to avoid confounding effects with female mating history. Each female was tested
196 during four consecutive days during daytime activity period (10:00-17:00 hours), at the same
197 hour of the day for all four trials. In general, each male was tested with only one female but,
198 because of difficulties with pooling similar males in quartets, 24 males participated to two
199 different quartets and were thus presented to two females (12 in 2012 and 12 in 2013). For
200 these males, at least two days separated the two mating trials to avoid effects of sperm
201 depletion. A previous study showed that male mating history did not affect male willingness
202 to mate (Kaufman, Laloï & Le Galliard, unpub. data). We thus considered the two repeats of
203 the same male as independent observations, thus demanding caution during results
204 interpretation. Similarly, 3 females and 9 males participated to trials both in 2012 and 2013,
205 against different individuals each year. We also considered between-years trials of the same
206 individual as independent observations.

207 Immediately before each trial, we emptied the female's terrarium and separated it into
208 two compartments with a removable opaque wall. After treatment application, one male was
209 introduced in the compartment unoccupied by the female. During the behavioral trials, white
210 UV-enriched light was provided by two UV-B neon tubes positioned 70 cm above the ground
211 and heat was provided by two incandescent bulbs placed above each compartment. Room
212 temperature was maintained at 20-21°C. After 10 min of acclimation, one incandescent bulb
213 of 40 W was turned off, leaving only the bulb above the female's compartment turned on to
214 generate a thermal gradient, and the opaque wall was removed gently to start behavioral
215 interactions.

216 All trials were videotaped with a digital camera (Wat-902B, Watec Co., LTD, Japan)
217 until the end of the first copulation attempt if pairing was successful or until one hour in the
218 other case. Videos were analyzed later by a person blind to the experimental treatments.
219 Generally, males' and females' reproductive behaviors were consistent with those observed in
220 the wild (pers. obs.), that is that the male approached and attempted to bite the female at the
221 tip of the tail. Then, after successive bites, the male moved its grip up to the posterior part of
222 the female's abdomen. Once well positioned, the male wrapped itself around the female and
223 adjoined his cloaca to the female's cloaca, which marked the beginning of a "copulation"
224 (hereafter called, pairing event). On average, pairing events lasted $24:17 \pm 08:56$ min (range:
225 $02:45 - 56:53$ min). From the beginning of the sequence until copulation, females resisted
226 more or less to the males' mating attempts by successive bites or flips (the female rolled
227 violently on itself). Thus, to assess female resistance to mating and pre-copulatory female
228 mating behavior, we counted the numbers of bites and the presence of female flips (binary
229 variable, due to strong over-dispersion in the number of flips; mean = 2.64 ± 12.01 , range = 0-
230 121) from each trial. We also extracted the pairing success (the presence or absence of
231 copulation during trial) and the duration of pairing when mating was successful (the duration
232 from cloaca apposition to partners separation).

233 Females that performed flips bit males more often (Wilcoxon rank sum test, $p < 0.0001$,
234 24.8 bites versus 5.77) and males that did not mate were more often bitten by the female ($p =$
235 0.0001 , 17 bites versus 6.54). The number of female bites was not related to the duration of
236 copulation (Spearman's rank correlation, $\rho = 0.08$, $p = 0.33$). Two days after the last
237 behavioral trial and before releasing the males, we collected a small part of their tail tip (1
238 mm) to extract DNA and assess paternity. Once all trials were completed, females were
239 released in small outdoor mesocosms (1 m², two females) in order to facilitate their
240 monitoring throughout gestation, with food and water *ad libitum*.

241 *Paternity assignments*

242 We recaptured the females a few days before parturition and placed them in the same
243 laboratory conditions as before (see above). At the time of parturition, we counted the number
244 of live newborns, dead newborns, and aborted or unfertilized eggs of each clutch. Tissue
245 samples (tail tips or egg samples) were collected from all newborns and eggs as well as from
246 mothers and were stored in 70% ethanol. Females were then released in the outdoor
247 enclosures with their live newborns. Genomic DNA was extracted from all tissue samples
248 using the QIAquick 96 Purification Kit (QIAGEN) according to the manufacturer's
249 instructions. Individuals were genotyped using 5 microsatellite markers (Lv-3-19, Lv-4-72,
250 Lv-4-alpha, Lv-4-X, and Lv-4-115, Richard *et al.*, 2009). Samples were run on an ABI 3100
251 genetic analyzer (Applied Biosystems) with a Genescan 600 Liz size standard. Sample data
252 were analyzed using either Genemapper 4.1 or Strand (Toonen and Hughes 2001,
253 <http://www.vgl.ucdavis.edu/STRand>). We checked for perfect match between reproductive
254 items (newborns and eggs) and their mother, and then assessed paternities (no mismatch
255 between potential father and the reproductive item) using CERVUS (Kalinowski, Taper, &
256 Marshall, 2007). Two females did not mate during the behavioral trials. Genomic DNA could
257 be extracted for all items except for one juvenile and 10 potentially unfertilized eggs laid by
258 six females. During paternity assignment tests, we found a single candidate father for all
259 except 2 juveniles and 3 dead embryos for which no valid DNA profile was available. All
260 analyses were therefore performed on a total of 230 eggs and offspring successfully attributed
261 to a unique father.

262 *Statistical analyses*

263 We used R 3.4.4 software (R Development Core Team, 2017) to conduct all statistical
264 analyses. We first tested the effects of male UV treatment, study year, and trial order on the
265 behavior of females (N = 4 measures per female). To do so, we used linear mixed-effects

266 models that account for random intercept variation among females in the *lme4* (Bates *et al.*,
267 2015) and *nlme* packages (Pinheiro *et al.*, 2019). Generalized mixed-effects models (GLMM)
268 were implemented to analyze the number of bites (Poisson distribution, log link) and the
269 presence of flips and the pairing success (binomial distribution, logit link) using the *glmer*
270 procedure. A linear mixed-effects model (LMM) was used to analyze the duration of
271 copulation using the *lme* procedure. All initial, full models included fixed, additive effects of
272 year, trial order (categorical factor), male UV treatment as well as their two-way and three-
273 way interactions. In addition, female body size (SVLf) and male head size (PC1) were
274 included as covariates. Model assumptions were checked prior to model selection, using tests
275 of goodness-of-fit (GLMM) and residual homoscedasticity and normality (LMM). To fulfil
276 the goodness-of-fit test, we calculated a transformed aggression score by binning the range of
277 number of female bites in 20 equally spaced breaks (similar results were obtained with 15-25
278 bins). Model parameters were estimated with a maximum likelihood approach and non-
279 significant effects were tested using likelihood ratio tests (Bolker *et al.*, 2009). Whenever test
280 statistics were borderline, we confirmed the strength of the effect by a parametric bootstrap
281 procedure of nested models ($n=1,000$ simulations) using the *PBmodcom* procedure
282 implemented in the *pbkrtest* package (Halekoh & Højsgaard, 2014). For the number of female
283 bites, we performed post-hoc Tukey tests to assess differences among the four trials.

284 Using generalized linear models, we further analyzed the effects of male UV
285 treatment, study year, and trial order on male mating success including the proportion of
286 fertilized eggs (i.e., fertilization success) and the total number of viable offspring sired by the
287 same male (hereafter referred to as total fitness). For fertilization success, we analyzed the
288 probability to sire at least one egg instead of the proportion of fertilized eggs because this
289 variable conformed better to a binomial distribution. Results were qualitatively similar in both
290 cases however. To analyze fertilization success, we used a logistic regression (logit link,

291 binomial errors) with the *glm* procedure (Venable & Ripley, 2002). Because of an excess of
292 zero, we analyzed the total male fitness using a zero-inflated model with the *zeroinfl*
293 procedure from the *pscl* package (Zeileis, Kleiber, & Jackman, 2008). This procedure allows
294 fitting a two-component mixture model combining a point mass at zero with a binary
295 modelling of unobserved state (zero vs. count, logit link and binomial errors) and a Poisson
296 distribution (log link, Poisson errors). For fertilization success, the initial model further
297 included additive effects of the number of males that mated with the female and the female's
298 clutch size, and trial order was replaced by male mating rank. The male mating rank excludes
299 records for which males did not mate and therefore describes better post-copulatory
300 mechanisms than trial order. Goodness-of-fit tests revealed that all initial models fitted
301 adequately the data. All minimum adequate models were then obtained by backward
302 elimination of non-significant terms. Estimates (hereafter named β) are provided with
303 standard errors unless otherwise stated.

304 *Ethical note*

305 All procedures comply with all laws on animal experimentation in France and Europe, and
306 were approved by authorization Ce5/2011/024.

307 **Results**

308 *Female resistance behavior prior to pairing*

309 The number of female bites ranged from 0 to 76 (mean = 9.7 ± 14.4 SD) and was best
310 predicted by the female mating history (trial order, likelihood ratio test: $df = 3$, $\chi^2 = 167.03$, p
311 < 0.0001), male UV treatment ($df = 1$, $\chi^2 = 4.48$, $p = 0.03$; parametric bootstrap test, $p =$
312 0.047), and study year ($df = 1$, $\chi^2 = 4.16$, $p = 0.04$). Male head size also had near-significant
313 positive effects ($df = 1$, $\chi^2 = 3.59$, $p = 0.06$, $\beta = 0.11 \pm 0.06$). Females were less aggressive
314 during the two first trials and bit on average about four times more during the two last trials
315 (Figure 1A). Post-hoc Tukey tests on trial order revealed that female bit more during the

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316 fourth trial than any other trial ($p < 0.01$ for each pairwise comparison), and more during the
317 third trial than during the two first ($p < 0.01$ for each pairwise comparison), but there was no
318 difference between the two first trials ($p = 0.97$). In addition, females significantly bit more in
319 2013 than in 2012 ($\beta = 0.32 \pm 0.15$) and UV-reduced males received more bites than control
320 males on average ($\beta = 0.17 \pm 0.07$; control = 8.9 ± 1.20 , UV-reduced = 10.5 ± 1.61 ; Figure
321 1B). The occurrence of female flips was not influenced by male UV treatment ($df = 1, \chi^2 =$
322 $0.50, p = 0.48$) and male head size ($df = 1, \chi^2 = 0.23, p = 0.63$). Occurrence of female flips
323 increased dramatically during the fourth mating trial ($df = 3, \chi^2 = 20.41, p = 0.01$, Figure 1C)
324 and was slightly higher in 2013 than in 2012 ($df = 1, \chi^2 = 4.24, p = 0.04, \beta = 0.91 \pm 0.44$).

325 *Pairing behavior*

326 During the behavioral trials, 2 females did not mate with any males (4 %), 3 females mated
327 with only one male (5 %), 11 females with two males (21 %), 24 females with three males (47
328 %) and 12 females with four males (23 %). In addition, 45 females mated during the first trial
329 (87%), 44 during the second (85%), 34 during the third (65%), and 22 during the fourth trial
330 (42%). Pairing success was influenced by trial order ($df = 3, \chi^2 = 29.6, p < 0.01$, Figure 2A)
331 and tended to be higher in 2013 than in 2012 ($df = 1, \chi^2 = 3.53, p = 0.06, 2013: \beta = 0.74 \pm$
332 0.40). Pairing occurred on average in more than 80% of the interactions during the first and
333 the second behavioral trials, but this dropped down to ca. 70% during the third trial and to ca.
334 40% during the fourth trial. Pairing success was not influenced by male UV treatment ($df = 1,$
335 $\chi^2 = 1.41, p = 0.23$, Figure 2) but increased slightly with male head size ($df = 1, \chi^2 = 3.83, p =$
336 $0.05, \beta = 0.39 \pm 0.20$). When pairing was successful ($N = 141$), the duration of copulation
337 (mean = $1444 \text{ sec} \pm 510 \text{ SD}$, range = 121-2881 sec) was not predicted by trial order ($F_{3,87} =$
338 $0.68, p = 0.56$) nor male head size ($F_{1,89} = 0.93, p = 0.30$). Instead, copulation duration was
339 influenced by the two-way interaction between study year and male UV treatment ($F_{1,89} =$
340 $6.73, p = 0.01$). In 2012, there was no effect of male UV treatment on copulation duration ($\beta =$

Female mate choice for UV reflectance

341 -160 sec \pm 126.3, $t = -1.27$, $p = 0.21$), but a drastic drop in copulation duration of UV-control
342 males occurred in 2013. As a result, copulation was 25% shorter for UV-control males than
343 for UV-reduced males in 2013 ($\beta = 431$ sec \pm 166.2, $t = 2.6$, $p = 0.01$, Figure 2B).

344 *Male mating success*

345 Paternity assignment tests showed that, among females paired with at least one male ($N = 50$),
346 8 did not produce any egg (16 %), 1 produced one egg (2 %), 14 produced from 2 to 4 eggs
347 (29 %), and 27 females produced from 5 to 8 eggs (53 %). Mated females that did not produce
348 any egg most probably failed to ovulate because they did not significantly increase body mass
349 (pers. obs.). Clutch size was not correlated with female body size (Pearson's product-moment
350 correlation test, $r = 0.06$, $p = 0.68$). Among the 47 females paired with at least two males, 12
351 females (25 %) were polyandrous and one clutch was sired by three different males.

352 The probability to sire at least one egg (our estimate of fertilization success) was best
353 predicted by a three-way interaction between study year, male mating rank and male UV
354 treatment (binomial regression, $df = 3$, $\chi^2 = 10.3$, $p = 0.02$) and by the number of mating ($df =$
355 1 , $\chi^2 = 4.27$, $p = 0.04$, negative effect), but not by male head size ($df = 1$, $\chi^2 = 0.91$, $p = 0.34$),
356 male throat UV coloration ($df = 1$, $\chi^2 = 0.17$, $p = 0.68$) or total clutch size ($df = 1$, $\chi^2 = 0.49$, p
357 $= 0.48$). Controlling for a positive effect of copulation duration on fertilization success ($df = 1$,
358 $\chi^2 = 15.4$, $p < 0.001$, $\beta = 0.99 \pm 0.29$) further improved the statistical significance of the three-
359 way interaction ($df = 3$, $\chi^2 = 11.5$, $p = 0.01$). Analysis of data from 2012 showed no effect of
360 male UV treatment and male mating rank on fertilization success (all $p > 0.25$): each male
361 fertilized on average 21.3 % of females' eggs. In 2013, fertilization success was affected by
362 the interaction between male mating rank and male UV treatment ($df = 3$, $\chi^2 = 15.75$, $p <$
363 0.01). Male fertilization success was similar for both UV-reduced and control males during
364 the first and second mating, but it dropped to zero during the third and fourth mating for UV-
365 reduced males (Figure 3).

366 We found no effect of the UV treatment and design factors on total male fitness (for
367 zero excess, effects of year: $df = 1, \chi^2 = 3.20, p = 0.07$; trial order: $df = 3, \chi^2 = 4.25, p = 0.23$;
368 male UV treatment: $df = 1, \chi^2 = 0.48, p = 0.49$; for count data, effects of year: $df = 1, \chi^2 =$
369 $0.21, p = 0.64$; trial order: $df = 3, \chi^2 = 0.88, p = 0.83$; male UV treatment: $df = 1, \chi^2 = 1.15, p =$
370 0.28). Male total fitness was not influenced by throat UV coloration (all $p > 0.16$), but it
371 increased with male head size (zero excess: $\chi^2 = 0.41, p = 0.52$, count: $\chi^2 = 7.01, p = 0.01, \beta =$
372 0.21 ± 0.08).

373 **Discussion**

374 Our study provides evidence suggesting that females can exert subtle mate preference (as
375 defined in Edward, 2015) with respect to male UV coloration in common lizards. The effects
376 of male UV coloration on precopulatory mate preference, copulation duration and male
377 fertilization success were modulated by the female's mating history and the study year, and
378 did not lead to significant changes in male total fitness. Specifically, we found evidence that
379 females were biting UV-reduced males, males of the last two trials, and males presented in
380 2013 significantly more. As a result, pairing success decreased with females' mating history.
381 Thus, these results seem to indicate that female limit their number of sexual partners, which
382 supports the hypothesis that mating is costly for female common lizards (Fitze *et al.*, 2005;
383 White *et al.*, 2011).

384 *Pre-copulatory and copulatory behavior*

385 Our results revealed that females were significantly more aggressive towards UV-reduced
386 males than towards control males, and were also more aggressive during the second year of
387 the study and during the last two mating trials. This suggests that females were more reluctant
388 to mate with UV-reduced males in general (e.g. Laloï *et al.*, 2011), and with later presented
389 males. In addition, females were least aggressive towards their first mates, maybe to ensure
390 fertilization of their eggs, and became more aggressive towards the subsequent partners,

391 which supports the hypothesis of trading-up mate choice in common lizards (Jennions &
392 Petrie, 2000; Fitze *et al.*, 2010; Laloï *et al.*, 2011). However, the number of female flips was
393 not influenced by our UV treatments, suggesting that pre-copulatory mate choice based on
394 UV signals is subtle and may involve other parameters (e.g. other signals or cues).

395 During the second year of the study, females mated for shorter time with UV-control
396 males than UV-reduced males. This result is counter-intuitive since longer pairing is
397 associated with larger amount of inseminated sperm, which increases male mating success
398 (reviewed in Simmons, 2005). A possible hypothesis may be that females perceived UV-
399 control males as potentially more harmful, and shortening copulations with those males allow
400 females to gain direct benefits. However, while UV features have been shown to correlated
401 with bite force in wall lizards (Pérez i de Lanuza *et al.*, 2014), it does not seem to be the case
402 in *Z. vivipara*. Instead, UV features appear to correlate with male body size and sprint speed
403 (Bonnaffé *et al.*, 2018; unpublished results). Although we used a randomized experimental
404 design, this result, along with the absence of effects of UV reflectance on female flips, could
405 also be explained by the use of other signals modalities or cues by females, such as chemical
406 signals. If female mate choice is based on multiple signals in this species, as is the case in
407 other lacertid lizards (Kopena *et al.* 2011; but see Rodríguez-Ruiz *et al.*, 2019), the de-
408 correlation between UV signals and chemical signals may have somewhat confused the
409 females. Thus, females may prioritize male UV signals in some situations and male chemical
410 signals (or other signals or cues) in others.

411 Interestingly, year of study appears to be an important factor explaining our results.
412 Females were more aggressive and tried to escape more in 2013 than in 2012, and copulation
413 duration decreased in 2013 for UV-control males. These effects could have to do with the
414 males being smaller in 2013 than in 2012, making it easier for females to reject them.
415 Although the males were size-matched within quartets and with the female, and that our

416 analyses controlled for differences in males head size within both years, a difference in
417 absolute male body size between years could potentially explain our results in this case. Other
418 speculative arguments may involve the contribution of year-dependent factors such as yearly
419 climate variations in the enclosures leading to differences in reproductive timing, female
420 condition and/or receptivity. Conducting studies over multiple years have the advantage of
421 providing higher sample sizes and allow a mid- to long-term assessment of the effects being
422 studied. However, inter-annual differences may occur and complexify the results and their
423 interpretation. In our study, the effect of study year is complex to interpret but emphasizes the
424 subtlety of the effect of male UV signals on female mate choice in this species better than we
425 if we had used only one study year.

426 Our results add to a growing list of studies showing that male UV coloration can
427 influence some components of female pre-copulatory mate choice in many species of birds
428 (Bennett *et al.*, 1996, 1997; Andersson & Amundsen, 1997; Hunt *et al.*, 1999; Siitari *et al.*,
429 2002; Pearn, Bennett, & Cuthill, 2003; Zampiga, Gaibani, & Csermely, 2008; Leitão,
430 Monteiro, & Mota, 2014), fishes (Kodric-Brown & Johnson, 2002; Macías Garcia & De
431 Perera, 2002; Smith *et al.*, 2002; Cummings, Rosenthal, & Ryan, 2003; Cummings *et al.*,
432 2006; Boulcott, Walton, & Braithwaite, 2005; Rick, Modarressie, & Bakker, 2006), in one
433 species of amphibians (Secondi *et al.*, 2012), and in a few lizard species (Bajer *et al.*, 2010;
434 Olsson *et al.*, 2011; Lisboa *et al.*, 2017). Several studies failed to find conclusive effects of
435 male UV coloration on female mate choice (Hunt *et al.*, 2001; Ballentine & Hill, 2003;
436 Cummings *et al.*, 2003; Liu, Siefferman, & Hill, 2007; Kurvers *et al.*, 2010). It could indeed
437 be simply because UV-based female mate choice is absent in these cases, or because the
438 methodology used was not adequate to detect its presence (e.g. UV manipulation outside of
439 the natural range of variation - Andersson & Amundsen, 1997; Siitari *et al.*, 2002; Kurvers *et*

440 *al.* 2010). UV-based mate choice is perhaps more widespread than previously thought in
441 lizards, and in vertebrates in general.

442 Furthermore, most experiments assessed female mate choice using simultaneous
443 choice tests. These mate choice design consists in presenting simultaneously two or more
444 males, placed in individual boxes such that they do not see each other, to a female from which
445 they are separated by a thin filter. Such a design controls well for male-male interactions but
446 interferes with physical and chemical exchanges usually involved in mate selection
447 (Shackleton, Jennions, & Hunt, 2005). Yet, reproductive success of males is modulated by
448 their ability to control the mating behavioral process, especially in the context of sexual
449 conflict (Arnqvist & Rowe, 2005), to which a simultaneous mate choice design is blind. In
450 addition, these study designs can only detect mate choice when females actively choose one
451 male over another, but fail to identify more subtle mate choice processes such as female
452 resistance to mating, and do not address copulatory and post-copulatory selective processes
453 (Eberhard, 1996). Here, the UV manipulation affected female pre-copulatory and copulation
454 behaviors but not pairing success, perhaps because the outcome of female-male interactions
455 was to some extent under male control (Fitze *et al.*, 2005; Fitze & Le Galliard, 2008).
456 Moreover, sequential mate choice is likely to be the norm for many polyandrous species in
457 which females can rarely compare males simultaneously (Milinski & Bakker, 1992). On top
458 of this, study design preventing contacts between males and females assess the role of UV
459 signals in the absence of other signals that are potentially important and thus overestimate
460 their role. In contrast, allowing these contacts provides information on the true role of UV
461 signals in the presence of other signals or cues. We thus recommend a similar design with
462 direct physical interaction for future investigations of female mate choice based on male
463 ornaments in species in which mating occurs sequentially in nature.

464 *Effects of the UV manipulation on male mating success*

465 First of all, we found that only 12 females (25 %) were polyandrous and only one clutch was
466 sired by three different males. This is a relatively low degree of polyandry compared to
467 previous studies (e.g. Fitze *et al.*, 2005). Paternity analyses revealed that UV-reduced and
468 control males had similar fertilization success in the first year of study despite increased
469 female aggression towards UV-reduced males. In the second year of study, however, we
470 found that fertilization success was similar for both UV-reduced and control males when they
471 were the first or second mating partners of females, but it was much smaller for UV-reduced
472 males when they were one of the two last mating partners. This suggests that some form of
473 cryptic female preference (Eberhard, 1996) or differential allocation (Sheldon, 2000) based on
474 male UV coloration negatively skewed fertilization success of UV-reduced males in the
475 second year. In other words, females may be able to modulate, at least to some extent, the
476 fertilization process. For example, they may use differential allocation based on male UV
477 coloration; females would allocate more resources when they mate with UV-control males
478 because they appear as more attractive than UV-reduced males.

479 However, we found that male UV treatment did not relate to male total fitness, which
480 included all pre-copulatory and post-copulatory components of sexual selection, whereas
481 there was a slight positive effect of head size. It should be noted that our study design was not
482 well-suited to test for fitness differences among males, as only one female was presented to
483 each male, and males and females were size-matched. In these conditions, the effect of male
484 perceived or intrinsic quality on male total fitness depended largely on female clutch size.
485 When we included female clutch size in these models, it was the only significant explanatory
486 variable, masking the effect of head size (Appendix 2).

487 *Conclusion*

488 In summary, our study suggests that male UV coloration acts as visual signal on which
489 females rely before and after copulation. However, the role of UV coloration was not

490 consistent across study years and trial order, indicating that female mate preference is
491 complex and involves other parameters. Overall, this supports the idea that male UV
492 coloration indicates some aspects of male quality in this species. In addition, our results
493 suggest that females may be able to bias sperm use in favor of males with higher UV
494 reflectance. Finally, we advocate that adequate study design may reveal that UV-based female
495 mate preference is actually more widespread than previously thought in lizards, and in
496 polyandrous species in general.

497

498 **Research data**

499 The data used in this study will be made freely available on a public repository upon
500 acceptance for publication.

501

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- 748

749 **Figures legends**

750

751 **Figure 1.** Pre-mating behavioral responses of females to the manipulation of the male UV
752 throat coloration. Number of bites performed by females against males during each behavioral
753 trial increased in response to changes in trial order (A, from 1st to fourth behavioral trial) and
754 with experimental reduction of throat UV coloration (B). The occurrence of female flip
755 behavior increased during the last trial order independently from the male UV treatment (C).
756 Raw data are represented as means \pm SE.

757

758 **Figure 2.** Pairing success and duration in females according to the manipulation of the male
759 UV throat coloration. The pairing success decreased in response to changes in trial order
760 (from 1st to fourth behavioral trial) irrespective of male UV treatment (A). Pairing duration, a
761 good potential indicator of copulation duration, was influenced by experimental reduction of
762 male UV reflectance differently in 2012 (no significant effect) and in 2013 (significant
763 effect). Raw data are given as means \pm SE.

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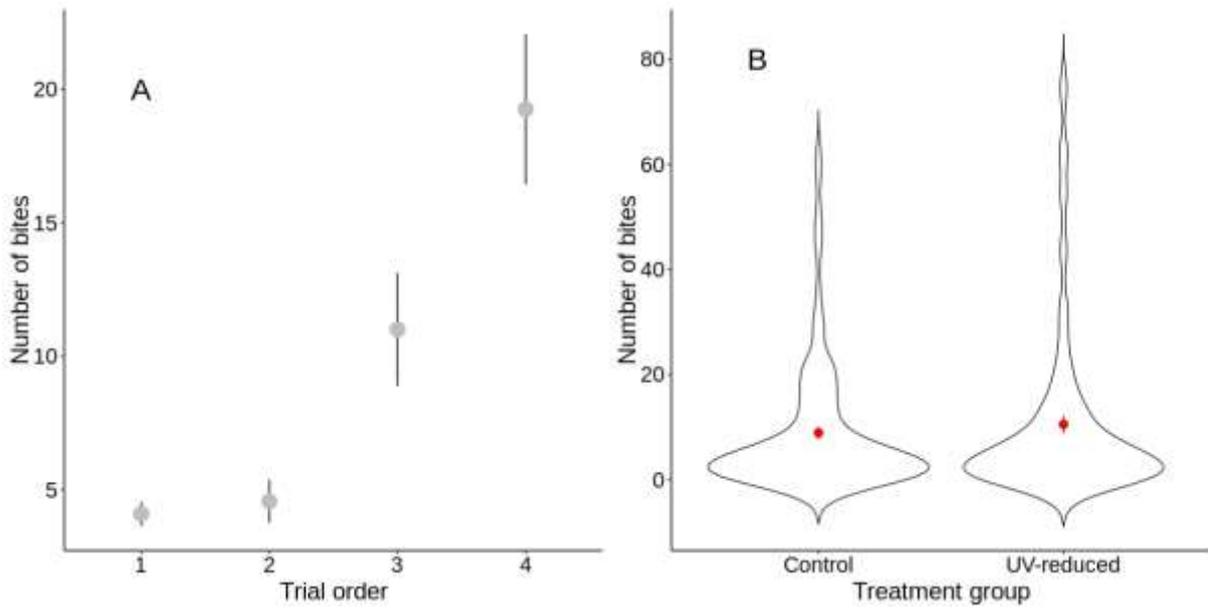
765 **Figure 3.** Proportion of fertilized eggs by males in 2012 and 2013 depending on their order of
766 presentation to females and their UV treatment. Data are given as means (\pm SE). Note that
767 fertilization success was quantified by the probability to sire at least one egg (see main text)
768 but results were qualitatively similar if we examined the proportion of fertilized eggs.

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770

771 **Figure 1**

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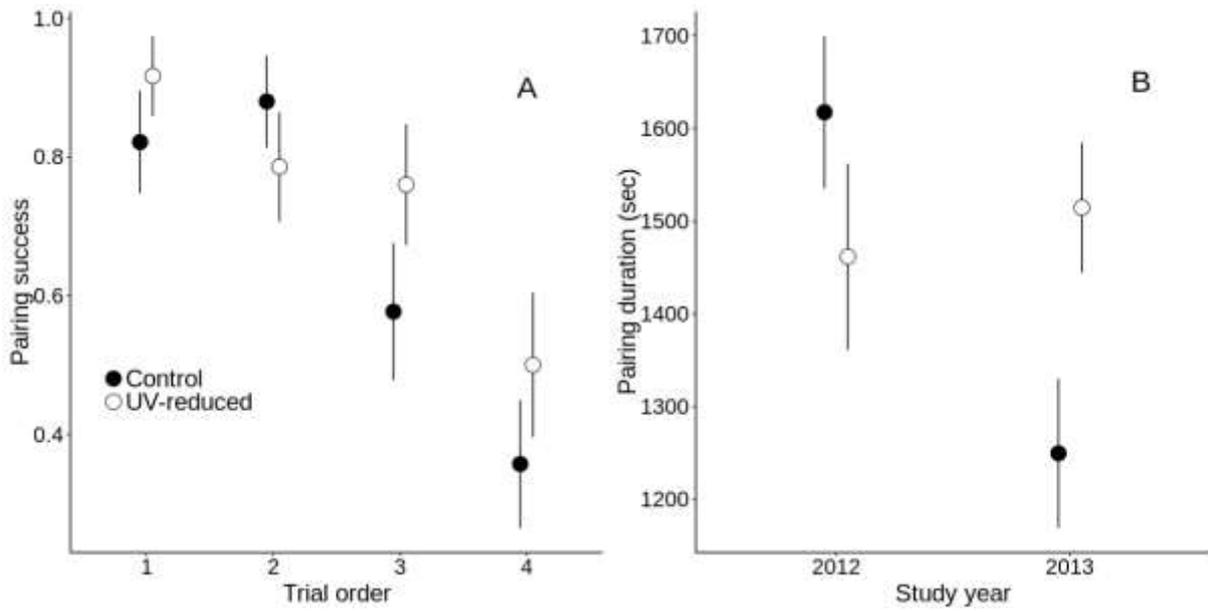
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777 **Figure 2**

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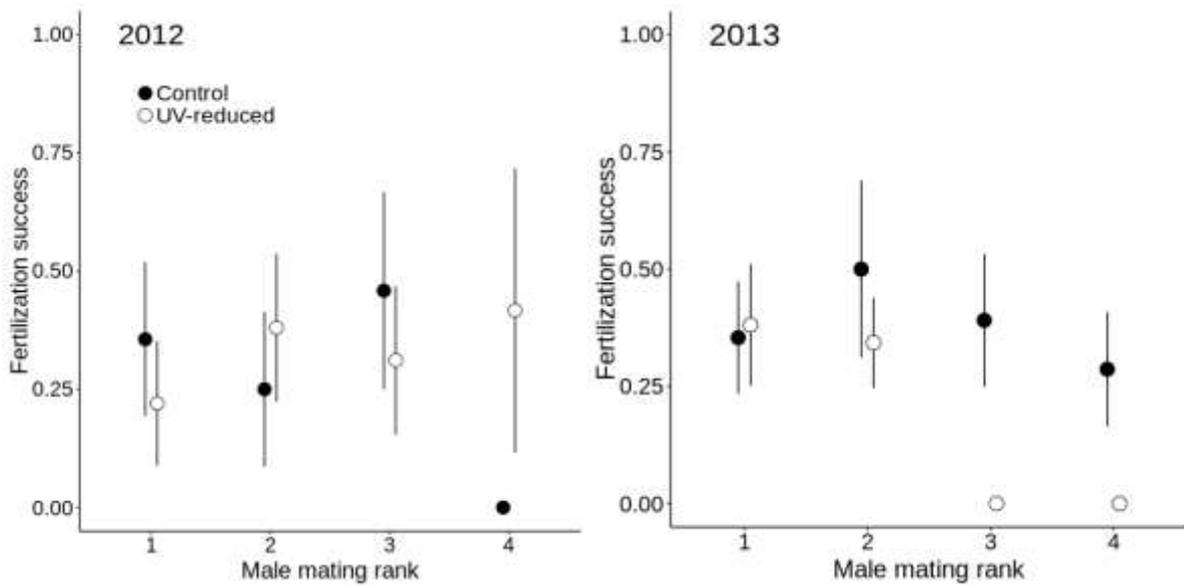
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782 **Figure 3**

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