

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

ARNAUD BADIANE^{1,*}, MÉLISSA MARTIN^{1,○}, SANDRINE MEYLAN¹,
MURIELLE RICHARD², BEATRIZ DECENCIÈRE FERRANDIÈRE³, and
JEAN-FRANÇOIS LE GALLIARD^{1,3}

¹Institut d'Écologie et des Sciences de l'Environnement (IEES), CNRS, IRD, INRA, Sorbonne Université, 4 Place Jussieu, 75005 Paris, France

²CNRS UMR 5321, Station d'Écologie Théorique et Expérimentale, Route du CNRS, 09200 Saint-Girons, France

³Centre de Recherche en Écologie Expérimentale et Prédicative (CEREPEP-Ecotron IleDeFrance), Ecole normale supérieure, CNRS, PSL Research University, UMS 3194, 11 chemin de Busseau, 77140 Saint-Pierre-lès-Nemours, France

Received 27 January 2020; revised 17 April 2020; accepted for publication 17 April 2020

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

ADDITIONAL KEYWORDS: colour signals – female choice – fertilization – paternity – post-copulatory selection – sexual selection – ultraviolet – *Zootoca vivipara*.

INTRODUCTION

Female mate choice is a major component of sexual selection that drives the evolution of male ornaments (Andersson, 1994). Choosing high quality males may increase female reproductive success (Andersson, 1994; Kokko *et al.*, 2003) by providing females with resources increasing their survival or fecundity (direct benefits: e.g. access to good territory, paternal care, protection against predators) or with alleles

enhancing the viability and/or attractiveness of their offspring [indirect benefits: “good genes” and “sexy sons” (Kirkpatrick & Ryan, 1991; Andersson, 1994; Johnstone, 1995)]. Females can assess males using signals that correlate consistently with male quality, and ultimately with those direct and indirect benefits (e.g. Cooper & Vitt, 1993; Welch *et al.*, 1998; Darragh *et al.*, 2017). In particular, many animal species exhibit colourful ornaments that convey an honest information about male age, phenotypic condition or genotypic quality (Senar, 2006; Bradbury & Vehrencamp, 2011; Weaver *et al.*, 2017).

*Corresponding author. E-mail: arnaud.badiane@gmail.com

Colour signals can be produced by the deposition of integumentary pigments (e.g. melanin and carotenoids), by coherent light-scattering nanostructures (i.e. structural coloration), or by a combination of both (Grether *et al.*, 2004; Shawkey & D'Alba, 2017; Fan *et al.*, 2019). Although the role of pigment-based colours in sexual selection has received much scientific attention (Svensson & Wong, 2011; Roulin, 2016), a growing body of work has emerged in the past two decades showing that structural colours, such as UV, could also function as sexual signals (Prum, 2006; Kemp *et al.*, 2012, 2015). Many vertebrate species display structural coloration that reflects light in the UV range (e.g. Andersson *et al.*, 1998; Siebeck, 2004; Ries *et al.*, 2008; Badiane *et al.*, 2018) and have a visual system sensitive to UV light (Bowmaker, 2008; Cronin & Bok, 2016). We have now good evidence that UV coloration can be sexually dichromatic (Hunt *et al.*, 1998; Names *et al.*, 2019) and can act as honest, condition-dependent indicator of male quality (e.g. Keyser & Hill, 1999, 2000; Griggio *et al.*, 2010; Pérez i de Lanuza *et al.*, 2014). Female mate choice based on male UV coloration has been demonstrated in birds (e.g. Hunt *et al.*, 1999), fishes (e.g. Kodric-Brown & Johnson, 2002), amphibians (e.g. Secondi *et al.*, 2012) and lizards (e.g. Bajer *et al.*, 2010). Most studies investigating the effect of UV coloration on female mate choice focused on pre-copulatory mechanisms whereas post-copulatory mechanisms remain rarely tested. Only Johnsen *et al.* (1998) investigated these aspects and found that the UV coloration of male bluethroats (*Luscinia s. svecica*) positively influenced social and genetic mate choice.

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

Here, we investigated whether male UV coloration influences behavioural mate preferences of females, mating behaviour and male mating success in the

common lizard *Z. vivipara*. Common lizards occupy overlapping home ranges (Massot *et al.*, 1992) and have a promiscuous mating system characterized by multiple matings in both sexes (Laloi *et al.*, 2004). Male common lizards exhibit a whitish coloration on their throat that strongly reflects UV light (Martin *et al.*, 2013; Bonnaffé *et al.*, 2018). Mating is under partial male control in common lizards (Fitze *et al.*, 2005; Fitze & Le Galliard, 2008); however, females can also select males by resisting mating and by sperm selection with multiple mating (Laloi *et al.*, 2004, 2011; Fitze *et al.*, 2005; Fitze *et al.*, 2010). During two successive years, we presented females sequentially with four different males with either a control or a reduced UV reflectance on their throat and belly, while controlling for other traits important for female mate choice. We quantified female resistance behaviour as well as pairing success and copulation duration to gain insights into pre-copulatory mechanisms of choice. To investigate post-copulatory mechanisms and quantify male mating success, we performed paternity analyses to assign offspring to males from both UV treatments. This study design allows us to test two main hypotheses. First, we hypothesize that females use male UV coloration to reject or accept a mating event with a male. If so, we expect females to resist more (biting more and flipping their body more often to escape) mating attempts initiated by UV-reduced males compared to UV-control males. Pairing success and copulation duration should also be higher for UV-control than for UV-reduced males. Second, we expect that, if cryptic female choice occurs, fertilization and reproductive success should be higher for UV-control males.

MATERIAL AND METHODS

STUDY SPECIES

The common lizard, *Z. vivipara*, is a small lacertid (45–70 mm in SVL) distributed across Eurasia. In our study site, animals reach sexual maturity at one or two years of age and mating takes place in April–May (Fitze *et al.*, 2005). Females are ovoviviparous and, after 2–3 months of gestation, give birth to 1–12 eggs depending on female age and body size (Massot *et al.*, 1992). Adult males have a whitish throat and a conspicuous belly ranging from yellow to dark red, interspersed with numerous black spots. Females display a duller ventral coloration ranging from cream to orange with fewer black spots than males (Bauwens, 1987; Cote *et al.*, 2008). In addition, the ventral coloration shows a secondary reflectance peak in the UV range, which is especially pronounced on the male's throat (Martin *et al.*, 2013). UV chroma of the throat and belly coloration increases with age and size in males (Bonnaffé *et al.*, 2018).

SAMPLING AND MORPHOMETRIC MEASUREMENTS

In 2012 and 2013, we captured by hand 184 adult males (85 in 2012 and 99 in 2013, 51–62 mm) and 52 adult females (24 in 2012 and 28 in 2013, 57–71 mm) at the Centre de Recherche en Ecologie Expérimentale et Prédictive (CEREEP-Ecotron IleDeFrance, 48°17'N, 2°41'E), where males and females had been maintained in separate 100-m² enclosures since 2011. Males were captured before their last moult at the onset of their sexual activity. Females were captured 10–15 days later once they emerged from wintering. Captures were carried out in mid-March 2012, and in early April 2013 due to annual differences in weather conditions and phenology.

We brought the lizards to the laboratory and measured their snout-vent length (SVL; ± 1 mm) and body mass (± 1 mg). We found no differences in female SVL (ANOVA, $F_{1,50} = 2.00$, $P = 0.16$) and body mass ($F_{1,50} = 3.14$, $P = 0.08$), nor in male body mass ($F_{1,181} = 0.38$, $P = 0.54$) between the two study years; however, males were larger in 2012 than in 2013 (SVL: $F_{1,181} = 5.25$, $P = 0.02$, $\beta = 0.72 \pm 0.32$ mm). We also obtained reflectance spectra of the throat and belly (two to three measures per location) of each male using a spectrophotometer [see [Martin et al. \(2013\)](#) for material details]. We then calculated brightness (total reflectance), yellow-red hue (wavelength of maximal reflectance), yellow-red saturation (difference between maximal reflectance over the range 450–700 nm and reflectance value at 450 nm divided by average reflectance over the range 300–700 nm), throat UV hue (wavelength of maximal reflectance between 300 nm and 400 nm) and throat UV chroma [proportion of the UV reflectance relative to the total reflectance, see [Martin et al. \(2013\)](#) for more details. The throat and ventral parts have different colours in this species (UV-white throats and yellow-red bellies), so we used the most adequate colour variable to characterize them. Males displayed higher throat UV hue (ANOVA, $F_{1,181} = 21.83$, $P < 0.0001$, $\beta = 3.29 \pm 0.70$), and lower yellow-red hue and yellow-red saturation in 2013 than in 2012 (yellow-red hue: $F_{1,181} = 7.27$, $P = 0.008$, $\beta = -5.94 \pm 2.2$; yellow-red saturation: $F_{1,181} = 21.83$, $P < 0.001$, $\beta = -0.08 \pm 0.03$) but had similar brightness ($F_{1,181} = 1.41$, $P = 0.24$) and throat UV chroma ($F_{1,181} = 1.14$, $P = 0.29$) between years.

We also quantified male head morphology using a digital caliper; we measured head length (from tip of the nose to the head skull-vertebral column articulation), head height (maximum height at the highest part posterior to orbita), head width (width at the maximum lateral extent), quadrate length, and coronoid length to the nearest 0.01 mm in all but one male. All measurements were highly correlated within the same individual (Spearman correlation,

$r > 0.33$, all $P < 0.001$) and most traits showed yearly variation in their mean similar to SVL. We therefore extracted a single metric of head size by a centred and scaled principal component analysis using the *dudi.pca* procedure in the *Ade4* package ([Chessel et al., 2004](#)). The first dominant axis (PC1) explained more than 62% of the inter-individual variation in head measurements and thus could be used as a head size metric. Individual scores for PC1 were positively correlated with body size ($r = 0.65$, $P < 0.0001$) but not with throat UV chroma ($r = -0.04$, $P = 0.54$). Males had smaller head size in 2013 than in 2012 ($F_{1,181} = 6.46$, $P = 0.01$, $\beta = -0.66 \pm 0.26$).

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

COLOUR MANIPULATION

To temporarily manipulate male UV reflectance within the natural range of variation ([Martin et al., 2015, 2016](#); [Names et al., 2019](#)), we used odourless UV-blocking (290–400 nm) inorganic agents (zinc oxide and titanium dioxide) mixed with a fat combination of petroleum jelly and liquid paraffin (6:4:50:40 for 100 g, respectively). Males of the control group were treated with the fat combination and males of the UV-reduced treatment were treated with the fat combination mixed with the inorganic agents. The combination was applied on the male's ventral skin with a soft paint brush from the tip of the nose to the anal plate. To validate our protocol, we measured the gular reflectance of randomly selected male lizards ($N = 7$ per group) before and after application of fat (control group) or of the UV-reducing treatment (UV-reduced group). Half an hour after the application, this treatment reduced UV reflectance within the natural range of variation of UV chroma (see [Supporting Information, Appendix S1](#)), and although the effect faded with time, it persisted for at least 2 h after application.

MATE CHOICE TRIALS

We designed sequential mating trials by pooling males into 52 quartets (24 in 2012 and 28 in 2013). This design mimicked the reproductive behaviour of common lizards, as highly mobile males likely

approach resident females in a sequential manner during the mating season. Males of the same quartet were matched by SVL (± 2 mm), body mass (± 600 mg) and gular, as well as ventral, coloration. For each quartet, two lizards were randomly attributed to the control group and to the UV-reduced group. We found no differences in morphology and coloration between UV-control and UV-reduced individuals prior to the experiment (Student's *t*-tests, all $P > 0.27$). Each male quartet was assigned to a single female according to their rank for SVL, such that larger females could mate with larger males (SVL difference between males and females, mean = 6.85 mm, range = 3–11 mm). This procedure avoided size mismatches so that we could focus on the role of UV coloration in mate choice, given the significant assortative mating by size in the common lizard (Richard *et al.*, 2005).

Each female encountered each of the four males in a random sequence of male UV treatments to avoid confounding effects with female mating history. Each female was tested during four consecutive days during the daytime activity period (10:00–17:00 h), at the same hour of the day for all four trials. In general, each male was tested with only one female but, because of difficulties with pooling similar males in quartets, 24 males participated to two different quartets and were thus presented to two females (12 in 2012 and 12 in 2013). For these males, at least 2 days separated the two mating trials to avoid effects of sperm depletion. A previous study showed that male mating history did not affect male willingness to mate (Kaufman JD, Laloï D, Le Galliard J-F, personal communication). We thus considered the two repeats of the same male as independent observations (i.e. as two different males), thus demanding caution during results interpretation. Similarly, three females and nine males participated in trials in both 2012 and 2013, against different individuals each year. We also considered between-years trials of the same individual as independent observations.

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

All trials were videotaped with a digital camera (Wat-902B, Watec Co., Ltd., Japan) until the end of

the first copulation attempt if pairing was successful or until 1 h in the other case. Videos were analysed later by a person blind to the experimental treatments. Generally, male and female reproductive behaviours were consistent with those observed in the wild (Le Galliard J-F, personal observation), that is that the male approached and attempted to bite the female at the tip of the tail. Then, after successive bites, the male moved its grip up to the posterior part of the female's abdomen. Once well positioned, the male wrapped itself around the female and adjoined his cloaca to the female's cloaca, which marked the beginning of a "copulation" (hereafter called the pairing event). On average, pairing events lasted $24:17 \pm 08:56$ min (range: 02:45–56:53 min). From the beginning of the sequence until copulation, females resisted more or less to the males' mating attempts by successive bites or flips (the female rolled violently on itself). Thus, to assess female resistance to mating and pre-copulatory female mating behaviour, we counted the numbers of bites and the presence of female flips (binary variable, due to strong over-dispersion in the number of flips; mean = 2.64 ± 12.01 , range = 0–121) from each trial. We also extracted the pairing success (the presence or absence of copulation during trial) and the duration of pairing when mating was successful (the duration from cloaca apposition to partner's separation).

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

PATERNITY ASSIGNMENTS

We recaptured the females a few days before parturition and placed them in the same laboratory conditions as before (see above). At the time of parturition, we counted the number of live newborns, dead newborns, and aborted or unfertilized eggs from each clutch. Tissue samples (tail tips or egg samples) were collected from all newborns and eggs, as well as from mothers, and were stored in 70% ethanol. Females were then released in the outdoor enclosures with their live newborns. Genomic DNA was extracted from all tissue samples using the QIAquick 96 Purification Kit (QIAGEN) according to the manufacturer's instructions. Individuals were genotyped using five

microsatellite markers [Lv-3–19, Lv-4–72, Lv-4-alpha, Lv-4-X and Lv-4–115 (Richard *et al.*, 2005)]. Samples were run on an ABI 3100 genetic analyser (Applied Biosystems) with a Genescan 600 Liz size standard. Sample data were analysed using either Genemapper v.4.1 or Strand [(Toonen and Hughes, 2001), <http://www.vgl.ucdavis.edu/STRand>]. We checked for perfect match between reproductive items (newborns and eggs) and their mother, and then assessed paternities (no mismatch between potential father and the reproductive item) using CERVUS (Kalinowski *et al.*, 2007). Two females did not mate during the behavioural trials. Genomic DNA could be extracted for all items except for one juvenile and ten potentially unfertilized eggs laid by six females. During paternity assignment tests, we found a single candidate father for all except two juveniles and three dead embryos for which no valid DNA profile was available. All analyses were therefore performed on a total of 230 eggs and offspring successfully attributed to a unique father.

STATISTICAL ANALYSES

We used R v.3.4.4 software (R Development Core Team, 2017) to conduct all statistical analyses. We first tested the effects of male UV treatment, study year, and trial order on the behaviour of females ($N = 4$ measures per female). To do so, we used linear mixed-effects models that account for random intercept variation among females in the *lme4* (Bates *et al.*, 2015) and *nlme* packages (Pinheiro *et al.*, 2019). Generalized mixed-effects models (GLMM) were implemented to analyse the number of bites (Poisson distribution, log link), the presence of flips, and pairing success (binomial distribution, logit link) using the *glmer* procedure. A linear mixed-effects model (LMM) was used to analyse the duration of copulation using the *lme* procedure. All initial, full models included the fixed, additive effects of year, trial order (categorical factor), and male UV treatment as well as the three pairwise interactions between these variables and the three-way interaction between these three variables. In addition, female body size (SVL_f) and male head size (PC1) were included as covariates. Model assumptions were checked prior to model selection, using tests of goodness-of-fit (GLMM) and residual homoscedasticity and normality (LMM). To fulfil the goodness-of-fit test, we calculated a transformed aggression score by binning the range of number of female bites in 20 equally spaced breaks (similar results were obtained with 15–25 bins). Model parameters were estimated with a maximum likelihood approach and non-significant effects were tested using likelihood ratio tests (Bolker *et al.*, 2009). Whenever test statistics were borderline, we confirmed the strength of the effect by a parametric bootstrap procedure of nested models ($N = 1000$ simulations)

using the *PBmodcom* procedure implemented in the *pbrtest* package (Halekoh & Højsgaard, 2014). For the number of female bites, we performed post-hoc Tukey tests to assess differences among the four trials.

Using generalized linear models, we further analysed the effects of male UV treatment, study year and trial order on male mating success including the proportion of fertilized eggs (i.e., fertilization success) and the total number of viable offspring sired by the same male (hereafter referred to as total fitness). For fertilization success, we analysed the probability to sire at least one egg instead of the proportion of fertilized eggs because this variable conformed better to a binomial distribution. Results were qualitatively similar in both cases however. To analyse fertilization success, we used a logistic regression (logit link, binomial errors) with the *glm* procedure (Venable & Ripley, 2002). Because of an excess of zero, we analysed the total male fitness using a zero-inflated model with the *zeroinfl* procedure from the *pscl* package (Zeileis *et al.*, 2008). This procedure allows fitting a two-component mixture model combining a point mass at zero with a binary modelling of unobserved state (zero vs. count, logit link and binomial errors) and a Poisson distribution (log link, Poisson errors). For fertilization success, the initial model further included additive effects of the number of males that mated with the female and the female's clutch size, and trial order was replaced by male mating rank. The male mating rank excludes records for which males did not mate and therefore better describes post-copulatory mechanisms than trial order. Goodness-of-fit tests revealed that all initial models adequately fitted the data. All minimum adequate models were then obtained by backward elimination of non-significant terms. Estimates (hereafter named β) are provided with standard errors unless otherwise stated.

ETHICAL NOTE

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

RESULTS

FEMALE RESISTANCE BEHAVIOUR PRIOR TO PAIRING

The number of female bites ranged from 0 to 76 (mean = 9.7 ± 14.4 SD) and was best predicted by the female mating history (trial order, likelihood ratio test: $df = 3$, $\chi^2 = 167.03$, $P < 0.0001$), male UV treatment ($df = 1$, $\chi^2 = 4.48$, $P = 0.03$; parametric bootstrap test, $P = 0.047$) and study year ($df = 1$, $\chi^2 = 4.16$, $P = 0.04$). Male head size also had near-significant positive effects ($df = 1$, $\chi^2 = 3.59$, $P = 0.06$, $\beta = 0.11 \pm 0.06$). Females were less aggressive

during the two first trials and bit on average about four times more during the two last trials (Fig. 1A). Post-hoc Tukey tests on trial order revealed that females bit more during the fourth trial than any other trial ($P < 0.01$ for each pairwise comparison), and more during the third trial than during the two first trials ($P < 0.01$ for each pairwise comparison); however, there was no difference between the two first trials ($P = 0.97$). In addition, females significantly bit more in 2013 than in 2012 ($\beta = 0.32 \pm 0.15$) and UV-reduced males received more bites than control males on average ($\beta = 0.17 \pm 0.07$; control = 8.9 ± 1.20 , UV-reduced = 10.5 ± 1.61 ; Fig. 1B).

The occurrence of female flips was not influenced by male UV treatment ($df = 1$, $\chi^2 = 0.50$, $P = 0.48$) and male head size ($df = 1$, $\chi^2 = 0.23$, $P = 0.63$). Occurrence of female flips increased dramatically during the fourth mating trial ($df = 3$, $\chi^2 = 20.41$, $P = 0.01$; Fig. 1C) and was slightly higher in 2013 than in 2012 ($df = 1$, $\chi^2 = 4.24$, $P = 0.04$, $\beta = 0.91 \pm 0.44$).

PAIRING BEHAVIOUR

During the behavioural trials, two females did not mate with any males (4%), three females mated with

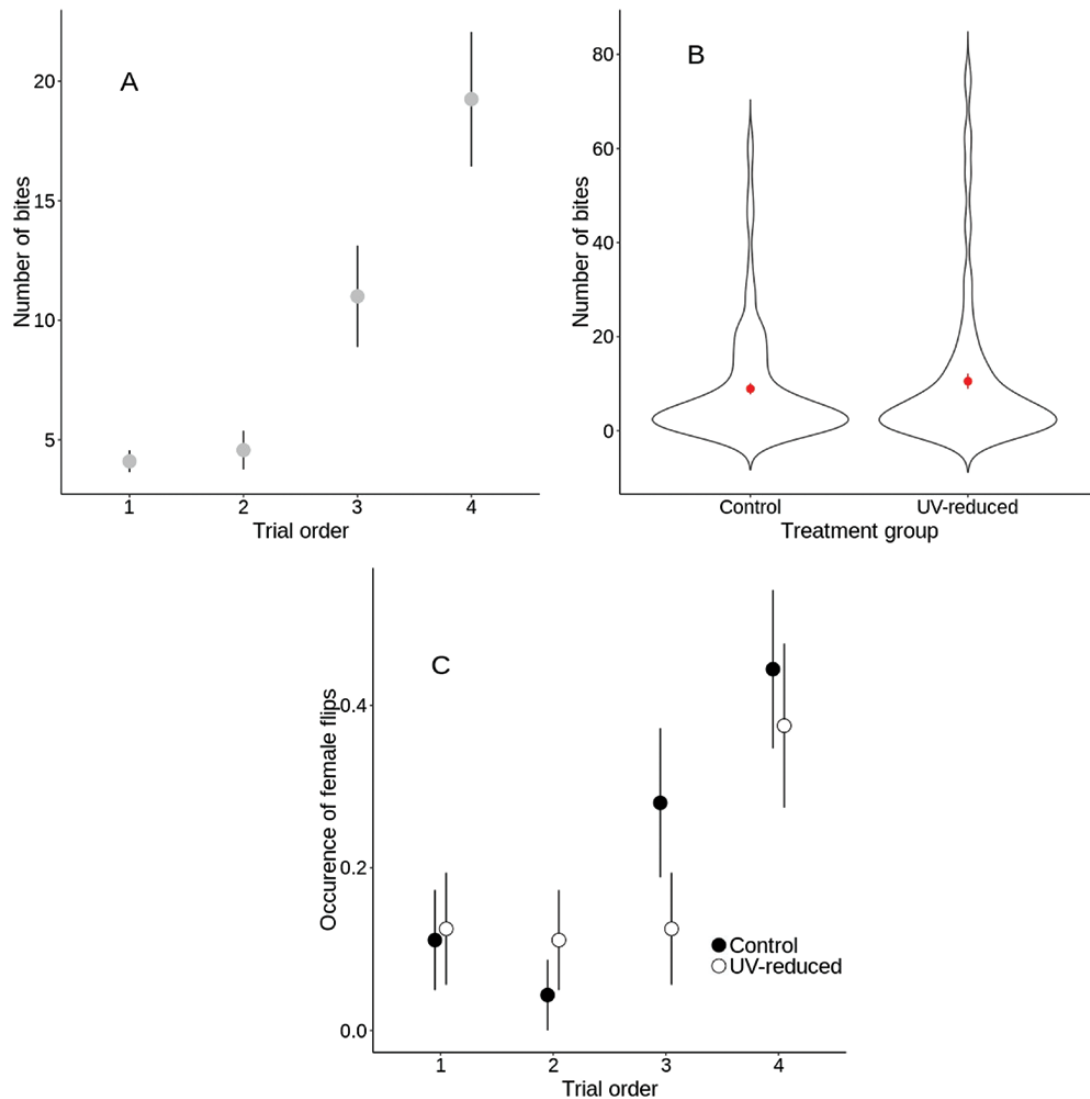


Figure 1. Pre-mating behavioural responses of females to the manipulation of the male UV throat coloration. Number of bites performed by females against males during each behavioural trial increased in response to changes in trial order (A), from first to fourth behavioural trial) and with experimental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

only one male (5%), 11 females with two males (21%), 24 females with three males (47%) and 12 females with four males (23%). In addition, 45 females mated during the first trial (87%), 44 during the second (85%), 34 during the third (65%), and 22 during the fourth (42%). Pairing success was influenced by trial order ($df = 3$, $\chi^2 = 29.6$, $P < 0.01$; Fig. 2A) and tended to be higher in 2013 than in 2012 ($df = 1$, $\chi^2 = 3.53$, $P = 0.06$, 2013: $\beta = 0.74 \pm 0.40$). Pairing occurred on average in more than 80% of the interactions during the first and the second behavioural trials; however, this dropped down to *c.* 70% during the third trial and to *c.* 40% during the fourth trial. Pairing success was not influenced by male UV treatment ($df = 1$, $\chi^2 = 1.41$, $P = 0.23$; Fig. 2) but increased slightly with male head size ($df = 1$, $\chi^2 = 3.83$, $P = 0.05$, $\beta = 0.39 \pm 0.20$). When pairing was successful ($N = 141$), the duration of copulation (mean = 1444 s \pm 510 SD, range = 121–2881 sec) was not predicted by trial order ($F_{3,87} = 0.68$, $P = 0.56$) nor male head size ($F_{1,89} = 0.93$, $P = 0.30$). Instead, copulation duration was influenced by the two-way interaction between study year and male UV treatment ($F_{1,89} = 6.73$, $P = 0.01$). In 2012, there was no effect of male UV treatment on copulation duration ($\beta = -160$ s \pm 126.3, $t = -1.27$, $P = 0.21$); however, a drastic drop in copulation duration of UV-control males occurred in 2013. As a result, copulation was 25% shorter for UV-control males than for UV-reduced males in 2013 ($\beta = 431$ s \pm 166.2, $t = 2.6$, $P = 0.01$; Fig. 2B).

MALE MATING SUCCESS

Paternity assignment tests showed that, among females paired with at least one male ($N = 50$), eight did not produce any egg (16%), one produced one egg (2%), fourteen produced from two to four eggs (29%), and 27 females produced from five to eight eggs (53%). Mated females that did not produce any eggs most probably failed to ovulate because they did not significantly increase body mass, as should be expected during gestation (Le Galliard J-F, personal observation). Clutch size was not correlated with female body size (Pearson's product-moment correlation test, $r = 0.06$, $P = 0.68$). Among the 47 females paired with at least two males, 12 females (25%) were polyandrous and one clutch was sired by three different males.

The probability to sire at least one egg (our estimate of fertilization success) was best predicted by a three-way interaction between study year, male mating rank and male UV treatment (binomial regression, $df = 3$, $\chi^2 = 10.3$, $P = 0.02$) and by the number of matings ($df = 1$, $\chi^2 = 4.27$, $P = 0.04$, negative effect), but not by male head size ($df = 1$, $\chi^2 = 0.91$, $P = 0.34$), male throat UV coloration ($df = 1$, $\chi^2 = 0.17$, $P = 0.68$) or total clutch size ($df = 1$, $\chi^2 = 0.49$, $P = 0.48$). Controlling for a positive effect of copulation duration on fertilization success ($df = 1$, $\chi^2 = 15.4$, $P < 0.001$, $\beta = 0.99 \pm 0.29$) further improved the statistical significance of the three-way interaction ($df = 3$, $\chi^2 = 11.5$, $P = 0.01$). Analysis of data from 2012 showed no effect of male UV treatment and

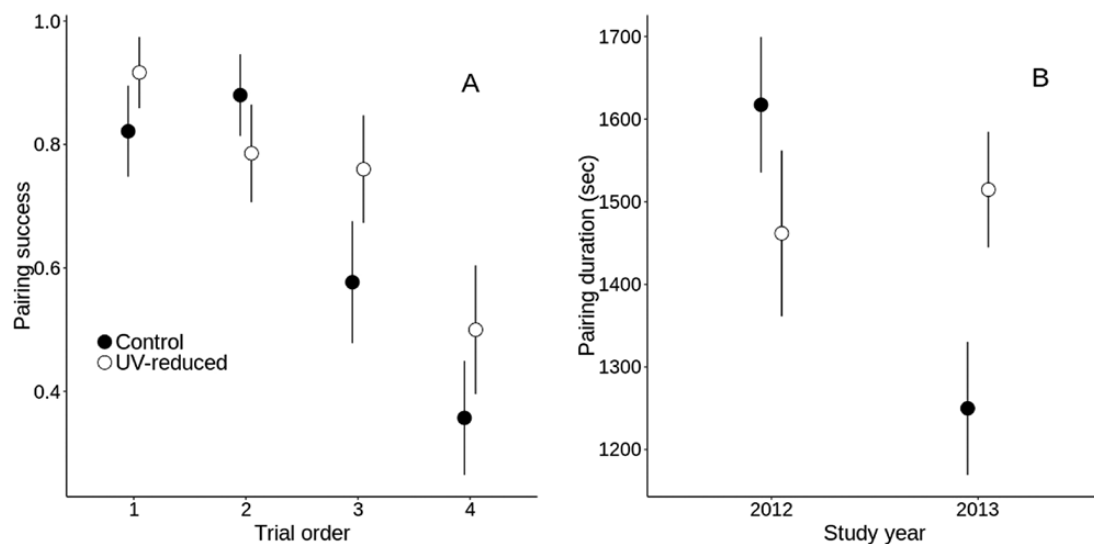


Figure 2. Pairing success and duration in females according to the manipulation of male UV throat coloration. The pairing success decreased in response to changes in trial order (from first to fourth behavioural trial) irrespective of male UV treatment (A). Pairing duration, a good potential indicator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

male mating rank on fertilization success (all $P > 0.25$): each male fertilized on average 21.3% of the females' eggs. In 2013, fertilization success was affected by the interaction between male mating rank and male UV treatment ($df = 3$, $\chi^2 = 15.75$, $P < 0.01$). Male fertilization success was similar for both UV-reduced and control males during the first and second mating; however, it dropped to zero during the third and fourth mating for UV-reduced males (Fig. 3).

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

DISCUSSION

Our study provides evidence suggesting that females can exert subtle mate preference [as defined in Edward (2015)] with respect to male UV coloration in common lizards. The effects of male UV coloration on precopulatory mate preference, copulation duration and male fertilization success were modulated by the female's mating history and the study year, and did not lead to significant changes in male total fitness.

Specifically, we found evidence that females were biting UV-reduced males, males of the last two trials, and males presented in 2013, significantly more. As a result, pairing success decreased with females' mating history. Thus, these results seem to indicate that females limit their number of sexual partners, which supports the hypothesis that mating is costly for female common lizards (Fitze *et al.*, 2005; White *et al.*, 2011).

PRE-COPULATORY AND COPULATORY BEHAVIOUR

Our results revealed that females were significantly more aggressive towards UV-reduced males than towards control males, and were also more aggressive during the second year of the study and during the last two mating trials. This suggests that females were more reluctant to mate with UV-reduced males in general (e.g. Laloï *et al.*, 2011), and with later presented males. In addition, females were least aggressive towards their first mates, maybe to ensure fertilization of their eggs, and became more aggressive towards the subsequent partners, which supports the hypothesis of trading-up mate choice in common lizards (Jennions & Petrie, 2000; Fitze *et al.*, 2010; Laloï *et al.*, 2011). However, the number of female flips was not influenced by our UV treatments, suggesting that pre-copulatory mate choice based on UV signals is subtle and may involve other parameters (e.g. other signals or cues).

During the second year of the study, females mated for a shorter time with UV-control males than UV-reduced males. This result is counter-intuitive

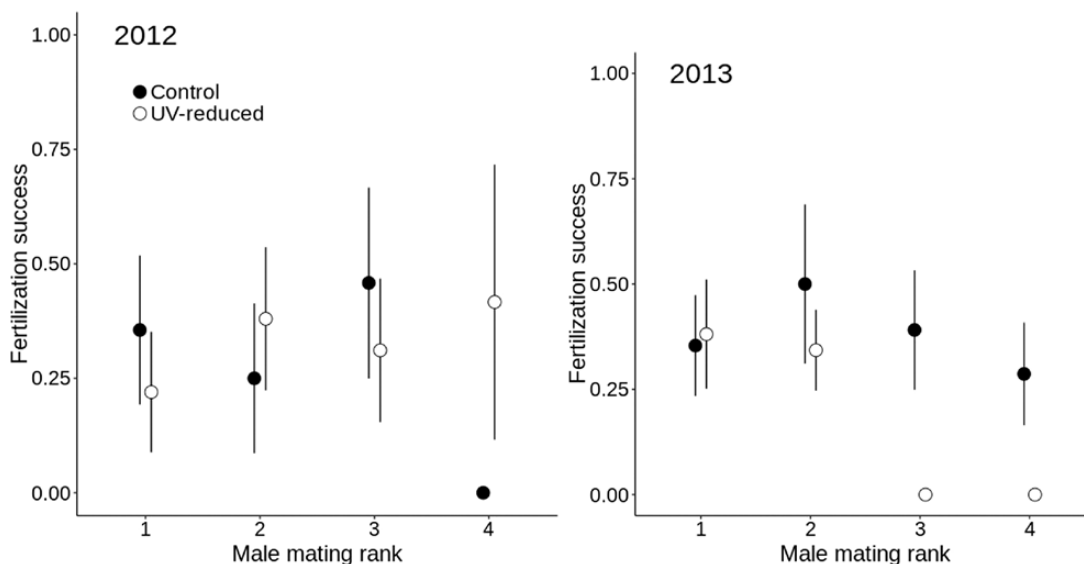


Figure 3. Proportion of eggs fertilized by males in 2012 and 2013 depending on their order of presentation to females and their UV treatment. Data are given as means (\pm SE). Note that fertilization success was quantified by the probability to sire at least one egg (see main text) but results were qualitatively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

since longer pairing is associated with larger amount of inseminated sperm, which increases male mating success [reviewed in [Simmons \(2005\)](#)]. A possible hypothesis may be that females perceived UV-control males as potentially more harmful, and shortening copulations with those males allow females to gain direct benefits. However, while UV features have been shown to correlated with bite force in wall lizards ([Pérez i de Lanuza *et al.*, 2014](#)), it does not seem to be the case in *Z. vivipara*. Instead, UV features appear to correlate with male body size and sprint speed ([Bonnaffé *et al.*, 2018](#); Badiane A, personal communication). Although we used a randomized experimental design, this result, along with the absence of effects of UV reflectance on female flips, could also be explained by the use of other signal modalities or cues by females, such as chemical signals. If female mate choice is based on multiple signals in this species, as is the case in other lacertid lizards [[Kopena *et al.* \(2011\)](#); but see [Rodríguez-Ruiz *et al.* \(2019\)](#)], the de-correlation between UV signals and chemical signals may have somewhat confused the females. Thus, females may prioritize male UV signals in some situations and male chemical signals (or other signals or cues) in others.

Interestingly, year of study appears to be an important factor explaining our results. Females were more aggressive and tried to escape more in 2013 than in 2012, and copulation duration decreased in 2013 for UV-control males. These effects could have to do with the males being smaller in 2013 than in 2012, making it easier for females to reject them. Although the males were size-matched within quartets and with the female, and that our analyses controlled for differences in males head size within both years, a difference in absolute male body size between years could potentially explain our results in this case. Other speculative arguments may involve the contribution of year-dependent factors such as yearly climate variations in the enclosures leading to differences in reproductive timing, female condition and/or receptivity. Conducting studies over multiple years has the advantage of providing higher sample sizes and allows a mid- to long-term assessment of the effects being studied. However, inter-annual differences may occur and complexify the results and their interpretation. In our study, the effect of study year is complex to interpret but emphasizes the subtlety of the effect of male UV signals on female mate choice in this species better than we if we had used only one study year.

Our results add to a growing list of studies showing that male UV coloration can influence some components of female pre-copulatory mate choice in many species of birds ([Bennett *et al.*, 1996, 1997](#); [Andersson & Amundsen, 1997](#); [Hunt *et al.*, 1999](#); [Siitari *et al.*, 2002](#); [Pearn *et al.*, 2003](#); [Zampiga *et al.*, 2008](#); [Leitão *et al.*, 2014](#)), fishes ([Kodric-Brown & Johnson, 2002](#); [Macías García & De Perera, 2002](#); [Smith *et al.*,](#)

[2002](#); [Cummings *et al.*, 2003, 2006](#); [Boulcott *et al.*, 2005](#); [Rick *et al.*, 2006](#)), in one species of amphibian ([Secondi *et al.*, 2012](#)) and in a few lizard species ([Bajer *et al.*, 2010](#); [Olsson *et al.*, 2011](#); [Lisboa *et al.*, 2017](#)). Several studies failed to find conclusive effects of male UV coloration on female mate choice ([Hunt *et al.*, 2001](#); [Ballentine & Hill, 2003](#); [Cummings *et al.*, 2003](#); [Liu *et al.*, 2007](#); [Kurvers *et al.*, 2010](#)). It could indeed be simply because UV-based female mate choice is absent in these cases, or because the methodology used was not adequate to detect its presence [e.g. UV manipulation outside of the natural range of variation ([Andersson & Amundsen, 1997](#); [Siitari *et al.*, 2002](#); [Kurvers *et al.*, 2010](#))]. UV-based mate choice is perhaps more widespread than previously thought in lizards, and in vertebrates in general.

Most experiments assessed female mate choice using simultaneous choice tests. These mate choice designs consist of simultaneously presenting two or more males, placed in individual boxes such that they do not see each other, to a female from which they are separated by a thin filter. Such a design controls well for male-male interactions but interferes with physical and chemical exchanges usually involved in mate selection ([Shackleton *et al.*, 2005](#)). Yet, reproductive success of males is modulated by their ability to control the mating behavioural process, especially in the context of sexual conflict ([Arnqvist & Rowe, 2005](#)), to which a simultaneous mate choice design is blind. In addition, these study designs can only detect mate choice when females actively choose one male over another, but fail to identify more subtle mate choice processes such as female resistance to mating, and do not address copulatory and post-copulatory selective processes ([Eberhard, 1996](#)). Here, the UV manipulation affected female pre-copulatory and copulation behaviours but not pairing success, perhaps because the outcome of female-male interactions was to some extent under male control ([Fitze *et al.*, 2005](#); [Fitze & Le Galliard, 2008](#)). Moreover, sequential mate choice is likely to be the norm for many polyandrous species in which females can rarely compare males simultaneously ([Milinski & Bakker, 1992](#)). On top of this, study design preventing contacts between males and females assess the role of UV signals in the absence of other signals that are potentially important. Therefore, the females have to make a decision based on the only signal available (i.e. UV signals), thus leading to an overestimation of the role and contribution of these signals during female mate choice. In contrast, allowing these contacts provides information on the true role of UV signals in the presence of other signals or cues. We thus recommend a similar design with direct physical interaction for future investigations of female mate choice based on male ornaments in species in which mating occurs sequentially in nature.

EFFECTS OF UV MANIPULATION ON MALE MATING SUCCESS

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

However, we found that male UV treatment did not relate to male total fitness, which included all pre-copulatory and post-copulatory components of sexual selection, whereas there was a slight positive effect of head size. It should be noted that our study design was not well-suited to test for fitness differences among males, as only one female was presented to each male, and males and females were size-matched. In these conditions, the effect of male perceived or intrinsic quality on male total fitness depended largely on female clutch size. When we included female clutch size in these models, it was the only significant explanatory variable, masking the effect of head size ([Supporting Information, Appendix S2](#)).

CONCLUSION

In summary, our study suggests that male UV coloration acts as visual signal on which females rely before and after copulation. However, the role of UV coloration was not consistent across study years and trial order, indicating that female mate preference is complex and involves other parameters. Overall, this supports the idea that male UV coloration indicates some aspects of male quality in this species. In addition, our results suggest that females may be able to bias sperm use in favour of males with higher UV reflectance. Finally, we advocate that adequate study design may reveal that

UV-based female mate preference is actually more widespread than previously thought in lizards, and in polyandrous species in general.

ACKNOWLEDGMENTS

We are very grateful to Amandine Boniface, Chloé Vilette and Samuel Perret for their help in the laboratory. This research was supported by the Centre National de la Recherche Scientifique (CNRS). This work further benefited from technical and human resources provided by CEREEP-Ecotron IleDeFrance (CNRS/ENS UMS 3194) as well as financial support from the Regional Council of Ile-de-France under the DIM Program R2DS bearing the reference I-05-098/R. It has received a support under the program “Investissements d’Avenir” launched by the French government and implemented by ANR with the reference ANR-11-INBS-0001 AnaEE France and ANR-10-IDEX-0001-02 PSL. We thank Simon Baeckens and one anonymous reviewer for their comments on the manuscript.

REFERENCES

- Andersson M. 1994.** *Sexual selection*. Princeton: Princeton University Press.
- Andersson S, Amundsen T. 1997.** Ultraviolet colour vision and ornamentation in bluethroats. *Proceedings of the Royal Society B: Biological Sciences* **264**: 1587–1591.
- Andersson S, Örnborg J, Andersson M. 1998.** Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society B: Biological Sciences* **265**: 445–450.
- Arnqvist G, Rowe L. 2005.** *Sexual conflict*. Princeton: Princeton University Press.
- Badiane A, Carazo P, Font E. 2018.** Colouration in male blue-throated keeled lizards (*Algyroides nigropunctatus*): evidence for ultraviolet reflectance of throat and lateral patches. *Herpetological Journal* **28**: 39–42.
- Bajer K, Molnár O, Torok J, Herczeg G. 2010.** Female European green lizards (*Lacerta viridis*) prefer males with high ultraviolet throat reflectance. *Behavioral Ecology and Sociobiology* **64**: 2007–2014.
- Bajer K, Molnár O, Török J, Herczeg G. 2011.** Ultraviolet nuptial colour determines fight success in male European green lizards (*Lacerta viridis*). *Biology Letters* **7**: 866–868.
- Ballentine B, Hill GE. 2003.** Female mate choice in relation to structural plumage coloration in blue grosbeaks. *The Condor* **105**: 593–598.
- Bates D, Maechler M, Bolker B, Walker S. 2015.** Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.
- Bauwens D. 1987.** Sex recognition by males of the lizard *Lacerta vivipara*: an introductory study. *Amphibia-Reptilia* **8**: 49–57.

- Badiane A, Martin M, Meylan S, Richard M, Decenci re Ferrandi re B, Le Galliard J-F. 2020.** Dataset: Male ultraviolet reflectance and female mating history influence female mate choice and male mating success in a polyandrous lizard. *Zenodo* doi:10.5281/zenodo.3757751
- Bennett ATD, Cuthill IC, Partridge JC, Maier EJ. 1996.** Ultraviolet vision and mate choice in zebra finches. *Nature* **380**: 433–435.
- Bennett ATD, Cuthill IC, Partridge JC, Lunau K. 1997.** Ultraviolet plumage colors predict mate preferences in starlings. *Proceedings of the National Academy of Sciences of the USA* **94**: 8618–8621.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS. 2009.** Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**: 127–135.
- Bonnaff  W, Mugabo M, Le Galliard J, Martin M, Meylan S. 2018.** Ontogenetic trajectories of body coloration reveal its function as a multicomponent nonsenescent signal. *Ecology and Evolution* **8**: 12299–12307.
- Boulcott PD, Walton K, Braithwaite VA. 2005.** The role of ultraviolet wavelengths in the mate-choice decisions of female three-spined sticklebacks. *Journal of Experimental Biology* **208**: 1453–1458.
- Bowmaker JK. 2008.** Evolution of vertebrate visual pigments. *Vision Research* **48**: 2022–2041.
- Bradbury JW, Vehrencamp SL. 2011.** *Principles of animal communication*. Sunderland: Sinauer Association Press.
- Chessel D, Dufour AB, Thioulouse J. 2004.** The ade4 package - I: one-table methods. *R News* **4**: 5–10.
- Cooper WE, Vitt LJ. 1993.** Female mate choice of large male broad-headed skinks. *Animal Behaviour* **45**: 683–693.
- Cote J, Le Galliard JF, Rossi JM, Fitze PS. 2008.** Environmentally induced changes in carotenoid-based coloration of female lizards: a comment on Vercken *et al.* *Journal of Evolutionary Biology* **21**: 1165–1172.
- Cronin TW, Bok MJ. 2016.** Photoreception and vision in the ultraviolet. *Journal of Experimental Biology* **219**: 2790–2801.
- Cummings ME, Rosenthal GG, Ryan MJ. 2003.** A private ultraviolet channel in visual communication. *Proceedings of the Royal Society B: Biological Sciences* **270**: 897–904.
- Cummings ME, De Le n FJG, Mollaghan DM, Ryan MJ. 2006.** Is UV ornamentation an amplifier in swordtails? *Zebrafish* **3**: 91–100.
- Darragh K, Vanjari S, Mann F, Gonzalez-Rojas MF, Morrison CR, Salazar C, Pardo-Diaz C, Merrill RM, McMillan WO, Schulz S, Jiggins CD. 2017.** Male sex pheromone components in *Heliconius* butterflies released by the androconia affect female choice. *PeerJ* **5**: e3953.
- Eberhard WG. 1996.** *Female control: sexual selection by cryptic female choice*. Princeton: Princeton University Press.
- Edward DA. 2015.** The description of mate choice. *Behavioral Ecology* **26**: 301–310.
- Fan M, D'Alba L, Shawkey MD, Peters A, Delhey K. 2019.** Multiple components of feather microstructure contribute to structural plumage colour diversity in fairy-wrens. *Biological Journal of the Linnean Society* **128**: 550–568.
- Fitze PS, Le Galliard JF. 2008.** Operational sex ratio, sexual conflict and the intensity of sexual selection. *Ecology Letters* **11**: 432–439.
- Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J. 2005.** Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. *Evolution* **59**: 2451–2459.
- Fitze PS, Cote J, Clobert J. 2010.** Mating order-dependent female mate choice in the polygynandrous common lizard *Lacerta vivipara*. *Oecologia* **162**: 331–341.
- Font E, Molina-Borja M. 2004.** Ultraviolet reflectance of color patches in *Gallotia galloti* lizards from Tenerife, Canary islands, Vol. 8. In: P rez-Mellado V, Riera N, Perera A, eds. *The biology of lacertid lizards. Evolutionary and ecological perspectives*. Mao (Spain): Institut Menorqui d'Estudis, 201–221.
- Grether GF, Kolluru GR, Nersissian K. 2004.** Individual colour patches as multicomponent signals. *Biological Reviews* **79**: 583–610.
- Griggio M, Zanollo V, Hoi H. 2010.** UV plumage color is an honest signal of quality in male budgerigars. *Ecological Research* **25**: 77–82.
- Halekoh U, H jsgaard S. 2014.** A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models - the R package pbkrtest. *Journal of Statistical Software* **59**: 1–30.
- Hunt S, Bennett ATD, Cuthill IC, Griffiths R. 1998.** Blue tits are ultraviolet tits. *Proceedings of the Royal Society B: Biological Sciences* **265**: 451–455.
- Hunt S, Cuthill IC, Bennett ATD, Griffiths R. 1999.** Preferences for ultraviolet partners in the blue tit. *Animal Behaviour* **58**: 809–815.
- Hunt S, Cuthill IC, Bennett ATD, Church SC, Partridge JC. 2001.** Is the ultraviolet waveband a special communication channel in avian mate choice? *Journal of Experimental Biology* **204**: 2499–2507.
- Jennions MD, Petrie M. 2000.** Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* **75**: 21–64.
- Johnsen A, Andersson S, Ornborg J, Lifjeld JT. 1998.** Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: *Luscinia s. svecica*): a field experiment. *Proceedings of the Royal Society B: Biological Sciences* **265**: 1313–1318.
- Johnstone RA. 1995.** Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews* **70**: 1–65.
- Kalinowski ST, Taper ML, Marshall TC. 2007.** Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099–1106.
- Kemp DJ, Herberstein ME, Grether GF. 2012.** Unraveling the true complexity of costly color signaling. *Behavioral Ecology* **23**: 233–236.
- Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett ATD, Dyer AG, Hart NS, Marshall J, Whiting MJ. 2015.** An integrative framework for the

- appraisal of coloration in nature. *The American Naturalist* **185**: 705–724.
- Keyser AJ, Hill GE. 1999.** Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proceedings of the Royal Society B: Biological Sciences* **266**: 771–777.
- Keyser AJ, Hill GE. 2000.** Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behavioral Ecology* **11**: 202–209.
- Kirkpatrick M, Ryan MJ. 1991.** The evolution of mating preferences and the paradox of the lek.pdf. *Nature* **350**: 33–38.
- Kodric-Brown A, Johnson SC. 2002.** Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. *Animal Behaviour* **63**: 391–396.
- Kokko H, Brooks R, Jennions MD, Morley J. 2003.** The evolution of mate choice and mating biases. *Proceedings of the Royal Society B: Biological Sciences* **270**: 653–664.
- Kopena R, Martín J, López P, Herczeg G. 2011.** Vitamin E supplementation increases the attractiveness of males' scent for female European green lizards. *PLoS One* **6**: 2–6.
- Kurvers RHJM, Delhey K, Roberts ML, Peters A. 2010.** No consistent female preference for higher crown UV reflectance in blue tits *Cyanistes caeruleus*: a mate choice experiment. *Ibis* **152**: 393–396.
- Laloi D, Richard M, Lecomte J, Massot M, Clobert J. 2004.** Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Molecular Ecology* **13**: 719–723.
- Laloi D, Eizaguirre C, Fédérici P, Massot M. 2011.** Female choice for heterozygous mates changes along successive matings in a lizard. *Behavioural Processes* **88**: 149–154.
- Leitão AV, Monteiro AH, Mota PG. 2014.** Ultraviolet reflectance influences female preference for colourful males in the European serin. *Behavioral Ecology and Sociobiology* **68**: 63–72.
- Lisboa CMCA, Bajer K, Pessoa DMA, Huber MAA, Costa GC. 2017.** Female Brazilian whiptail lizards (*Cnemidophorus ocellifer*) prefer males with high ultraviolet ornament reflectance. *Behavioural Processes* **142**: 33–39.
- Liu M, Siefferman L, Hill GE. 2007.** An experimental test of female choice relative to male structural coloration in eastern bluebirds. *Behavioral Ecology and Sociobiology* **61**: 623–630.
- MacGregor HEA, While GM, Barrett J, Pérez i de Lanuza G, Carazo P, Michaelides S, Uller T. 2017.** Experimental contact zones reveal causes and targets of sexual selection in hybridizing lizards. *Functional Ecology* **31**: 742–752.
- Macías García C, De Perera TB. 2002.** Ultraviolet-based female preferences in a viviparous fish. *Behavioral Ecology and Sociobiology* **52**: 1–6.
- Martin M, Meylan S, Gomez D, Le Galliard JF. 2013.** Ultraviolet and carotenoid-based coloration in the viviparous lizard *Zootoca vivipara* (Squamata: Lacertidae) in relation to age, sex, and morphology. *Biological Journal of the Linnean Society* **110**: 128–141.
- Martin M, Meylan S, Perret S, Le Galliard JF. 2015.** UV coloration influences spatial dominance but not agonistic behaviors in male wall lizards. *Behavioral Ecology Sociobiology* **69**: 1483–1491.
- Martin M, Meylan S, Haussy C, Decencière B, Perret S, Le Galliard JF. 2016.** UV color determines the issue of conflicts but does not covary with individual quality in a lizard. *Behavioral Ecology* **27**: 262–270.
- Massot M, Clobert J, Pilorge T, Lecomte J, Barbault R. 1992.** Density dependence in the common lizard: demographic consequences of a density manipulation. *Ecology* **73**: 1742–1756.
- Milinski M, Bakker TCM. 1992.** Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proceedings of the Royal Society B: Biological Sciences* **250**: 229–233.
- Molnár O, Bajer K, Torok J, Herczeg G. 2012.** Individual quality and nuptial throat colour in male European green lizards. *Journal of Zoology* **287**: 233–239.
- Molnár O, Bajer K, Mészáros B, Torok J, Herczeg G. 2013.** Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton-Zuk hypothesis. *Naturwissenschaften* **100**: 551–558.
- Names G, Martin M, Badiane A, Le Galliard JF. 2019.** The relative importance of body size and UV coloration in influencing male-male competition in a lacertid lizard. *Behavioral Ecology and Sociobiology* **73**: 98.
- Olsson M, Andersson S, Wapstra E. 2011.** UV-deprived coloration reduces success in mate acquisition in male sand lizards (*Lacerta agilis*). *PLoS ONE* **6**: 3–6.
- Pearn SM, Bennett ATD, Cuthill IC. 2003.** The role of ultraviolet-A reflectance and ultraviolet-A-induced fluorescence in budgerigar mate choice. *Ethology* **109**: 961–970.
- Pérez i de Lanuza G, Carazo P, Font E. 2014.** Colours of quality: structural (but not pigment) coloration informs about male quality in a polychromatic lizard. *Animal Behaviour* **90**: 73–81.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2019.** *nlme: Linear and nonlinear mixed effects models*. R package version 3.1-141. Available at: <https://CRAN.R-project.org/package=nlme>
- Prum RO. 2006.** Anatomy, physics, and evolution of structural colours. In: McGraw KJ, ed. *Bird coloration. Vol. 1. Mechanisms and measurements*. Cambridge: Harvard University Press, 295–353.
- R Development Core Team. 2017.** *R: a language and environment for statistical computing*. Vienna, Austria: R foundation for statistical computing.
- Richard M, Lecomte J, De Fraipont M, Clobert J. 2005.** Age-specific mating strategies and reproductive senescence. *Molecular Ecology* **14**: 3147–3155.
- Rick IP, Modarressie R, Bakker TCM. 2006.** UV wavelengths affect female mate choice in three-spined sticklebacks. *Animal Behaviour* **71**: 307–313.
- Ries C, Spaethe J, Sztatecsny M, Strondl C, Hödl W. 2008.** Turning blue and ultraviolet: sex-specific colour change

- during the mating season in the Balkan moor frog. *Journal of Zoology* **276**: 229–236.
- Rodriguez-Ruiz G, Lopez P, Martin J. 2019.** Possible reproductive benefits to female Carpetan rock lizards of pre-sensory bias towards chemical signals. *Biological Journal of the Linnean Society* **127**: 787–799.
- Roulin A. 2016.** Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. *Biological Reviews* **91**: 328–348.
- Secondi J, Lepetz V, Théry M. 2012.** Male attractiveness is influenced by UV wavelengths in a newt species but not in its close relative. *PLoS ONE* **7**: 1–7.
- Senar JC. 2006.** Colour displays as intrasexual signals of aggression and dominance. In: McGraw KJ, Hill GE, eds. *Bird colouration. Vol. II. Function and evolution*. Cambridge: Harvard University Press, 87–136.
- Shackleton MA, Jennions MD, Hunt J. 2005.** Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology* **58**: 1–8.
- Shawkey MD, D’Alba L. 2017.** Interactions between colour-producing mechanisms and their effects on the integumentary colour palette. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**: 20160536.
- Sheldon BC. 2000.** Differential allocation: tests, mechanisms and implications. *Trends in Ecology and Evolution* **15**: 397–402.
- Siebeck UE. 2004.** Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour. *Animal Behaviour* **68**: 273–282.
- Siitari H, Honkavaara J, Huhta E, Viitala J. 2002.** Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. *Animal Behaviour* **63**: 97–102.
- Simmons LW. 2005.** The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Annual Review of Ecology, Evolution, and Systematics* **36**: 125–146.
- Smith EJ, Partridge JC, Parsons KN, White E, Cuthill IC, Bennett ATD, Church SC. 2002.** Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology* **13**: 11–19.
- Stapley J, Whiting MJ. 2006.** Ultraviolet signals fighting ability in a lizard. *Biology Letters* **2**: 169–172.
- Svensson PA, Wong BBM. 2011.** Carotenoid-based signals in behavioural ecology: a review. *Behaviour* **148**: 131–189.
- Thorpe RS, Richard M. 2001.** Evidence that ultraviolet markings are associated with patterns of molecular gene flow. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 3929–3934.
- Toonen RJ, Hughes S. 2001.** Increased throughput for fragment analysis on an ABI PRISM (R) automated sequencer using a membrane comb and STRand software. *Biotechniques* **31**: 1320–1324.
- Venable WN, Ripley BD. 2002.** *Modern applied statistics with S*. New York: Springer.
- Weaver RJ, Koch RE, Hill GE. 2017.** What maintains signal honesty in animal colour displays used in mate choice? *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **372**: 20160343.
- Welch AM, Semlitsch RD, Gerhardt HC. 1998.** Call duration as an indicator of genetic quality in male gray tree frogs. *Science* **280**: 1928–1930.
- White J, Richard M, Massot M, Meylan S. 2011.** Cloacal bacterial diversity increases with multiple mates: evidence of sexual transmission in female common lizards. *PLoS One* **6**: e22339.
- Whiting MJ, Stuart-Fox DM, O’Connor D, Firth D, Bennett NC, Blomberg SP. 2006.** Ultraviolet signals ultra-aggression in a lizard. *Animal Behaviour* **72**: 353–363.
- Zampiga E, Gaibani G, Csermely D. 2008.** Ultraviolet reflectance and female mating preferences in the common kestrel (*Falco tinnunculus*). *Canadian Journal of Zoology* **86**: 479–483.
- Zeileis A, Kleiber C, Jackman S. 2008.** Regression models for count data in R. *Journal of Statistical Software* **27**: 1–25.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Appendix S1. Experimental manipulation of throat coloration.

Appendix S2. Predictors of clutch size.

SHARED DATA

Data deposited in the public repository Zenodo ([Badiane et al., 2020](#)).