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

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Short title: UV reflectance influences female mate choice
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Abstract

Pre-copulatory female mate choice based on male ultraviolet (UV) coloration has been demonstrated in several vertebrate species, but 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 behavior, copulation behavior and assigned paternity to all offspring. Females were more aggressive towards UV-reduced males and, in one year, UV-reduced males had a lower probability of siring at least one egg (fertilization success) during the last mating trials. However, in one 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 vertebrates, and emphasize the importance to use a study design well adapted to the species reproductive behavior.

Keywords: Color 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, Semlitsch, & Gerhardt, 1998; Darragh et al., 2017). In particular, many animal species exhibit colorful ornaments that convey an honest information about male age, phenotypic condition, or genotypic quality (Senar, 2006; Bradbury & Vehrencamp, 2011; Weaver, Koch, & Hill, 2017).

Color 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, Kolluru & Nersissian, 2004; Shawkey & D’Alba, 2017; Fan et al., 2019). While the role of pigment-based colors 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 colors, such as ultraviolet, could also function as sexual signals (Prum 2006; Kemp et al. 2012, 2015). Many vertebrate species display structural coloration that reflects light in the ultraviolet (UV) range (e.g. Andersson, Örnborg, & Andersson, 1998; Siebeck, 2004; Ries et al., 2008; Badiane, Carazo, & Font, 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 act as honest, condition-dependent
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indicator of male quality (e.g. Keyser & Hill, 1999, 2000; Griggio, Zanollo, & Hoi, 2010; Pérez i de Lanuza, Carazo & Font, 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, Lepetz, & Théry, 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 while 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 ultraviolet color 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, Carazo, & Font, 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 European green lizards 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., 2010). However, none of these studies tested the influence of UV signaling on male mating success.

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

Materials and methods

Study species

The common lizard, Zootoca vivipara, is a small lacertid (45-70 mm) distributed across
Eurasia. In our study site, animals reach sexual maturity at one or two years of age and mating
takes place in 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.,
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In addition, the ventral coloration shows a secondary reflectance peak in the UV range, which is especially pronounced on males’ 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 183 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 were maintained in separate 100-m² enclosures since 2011. Males were captured before their last molt at the onset of their sexual activity. Females were captured 10-15 days later once they emerged from wintering. Captures occurred in mid-March 2012 but early April 2013 because of 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 (± 1mg). 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, but 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 (2-3 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 colors in this species (UV-white throats and yellow-red bellies), so we used the most adequate color variable to characterize them. Males displayed higher throat
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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 centered and scaled principal component analysis using the "dudi.pca" procedure in Ade4 package (Chessel, Dufour, & Thioulouse, 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 behavioral 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 behavioral 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
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hours a day. Food (crickets, Acheta domesticus) and water were provided ad libitum during
the experiment.

Color manipulation

To manipulate temporarily male UV reflectance within the natural range of variation (Martin
et al., 2015, 2016; Names et al. 2019), we used odorless UV-blocking (290-400 nm) inorganic
agents (zinc oxide and titan dioxide) mixed with a fat combination of petroleum jelly and
liquid paraffin (respectively, 6:4:50:40 for 100 g). Males of the control group were treated
with the fat combination and males of the UV-reduced treatment with the fat combination
mixed with the inorganic agents. The combination was applied on the males’ ventral skin with
a soft paintbrush 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 Appendix 1), and although the effect faded with time, it
persisted for at least two hours 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 behavior 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
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between males and females, mean = 6.85 mm, range = 3-11). 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 daytime activity period (10:00-17:00 hours), 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 two 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, Laloi & Le Galliard, unpub. data). We thus considered the two repeats of
the same male as independent observations, thus demanding caution during results
interpretation. Similarly, 3 females and 9 males participated to trials both in 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 behavioral 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 behavioral
interactions.
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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 one hour in the other case. Videos were analyzed later by a person blind to the experimental treatments. Generally, males’ and females’ reproductive behaviors were consistent with those observed in the wild (pers. obs.), 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, pairing event). On average, pairing events lasted 24:17 ± 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 behavior, 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 partners separation).

Females that performed flips bit males more often (Wilcoxon rank sum test, $p < 0.0001$, 24.8 bites versus 5.77) and males that did not mate were more often bitten by the female ($p = 0.0001$, 17 bites versus 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 behavioral 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, two females) in order to facilitate their monitoring throughout gestation, with food and water ad libitum.
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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 of 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 5 microsatellite markers (Lv-3-19, Lv-4-72, Lv-4-alpha, Lv-4-X, and Lv-4-115, Richard et al., 2009). Samples were run on an ABI 3100 genetic analyzer (Applied Biosystems) with a Genescan 600 Liz size standard. Sample data were analyzed using either Genemapper 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, Taper, & Marshall, 2007). Two females did not mate during the behavioral trials. Genomic DNA could be extracted for all items except for one juvenile and 10 potentially unfertilized eggs laid by six females. During paternity assignment tests, we found a single candidate father for all except 2 juveniles and 3 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 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 behavior 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 analyze the number of bites (Poisson distribution, log link) and the presence of flips and the pairing success (binomial distribution, logit link) using the glmer procedure. A linear mixed-effects model (LMM) was used to analyze the duration of copulation using the lme procedure. All initial, full models included fixed, additive effects of year, trial order (categorical factor), male UV treatment as well as their two-way and three-way interactions. In addition, female body size (SVLf) 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=1,000 simulations) using the PBmodcom procedure implemented in the pbkrtest 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 analyzed 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 analyzed 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 analyze fertilization success, we used a logistic regression (logit link,
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binomial errors) with the *glm* procedure (Venable & Ripley, 2002). Because of an excess of zero, we analyzed the total male fitness using a zero-inflated model with the *zeroinfl* procedure from the *pscl* package (Zeileis, Kleiber, & Jackman, 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 describes better post-copulatory mechanisms than trial order. Goodness-of-fit tests revealed that all initial models fitted adequately the data. All minimum adequate models were then obtained by backward elimination of non-significant terms. Estimates (hereafter named $\beta$) 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 behavior prior to pairing**

The number of female bites ranged from 0 to 76 (mean $=9.7 \pm 14.4$ 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 (Figure 1A). Post-hoc Tukey tests on trial order revealed that female bit more during the
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fourth trial than any other trial ($p < 0.01$ for each pairwise comparison), and more during the third trial than during the two first ($p < 0.01$ for each pairwise comparison), but 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 = $8.9 \pm 1.20$, UV-reduced = $10.5 \pm 1.61$; Figure 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 ($df = 3, \chi^2 = 20.41, p = 0.01$, Figure 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 behavior

During the behavioral trials, 2 females did not mate with any males (4 %), 3 females mated with 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 trial (42%). Pairing success was influenced by trial order ($df = 3, \chi^2 = 29.6, p < 0.01$, Figure 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 behavioral trials, but this dropped down to ca. 70% during the third trial and to ca. 40% during the fourth trial. Pairing success was not influenced by male UV treatment ($df = 1, \chi^2 = 1.41, p = 0.23$, Figure 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 = $1444 \text{ sec} \pm 510 \text{ SD}$, range = $121-2881 \text{ sec}$) 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 ($\beta =$
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-160 sec ± 126.3, \( t = -1.27, p = 0.21 \), but 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 (\( \beta = 431 \) sec ± 166.2, \( t = 2.6, p = 0.01 \), Figure 2B).

**Male mating success**

Paternity assignment tests showed that, among females paired with at least one male (N = 50), 8 did not produce any egg (16%), 1 produced one egg (2%), 14 produced from 2 to 4 eggs (29%), and 27 females produced from 5 to 8 eggs (53%). Mated females that did not produce any egg most probably failed to ovulate because they did not significantly increase body mass (pers. obs.). 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 mating (\( df = 1, \chi^2 = 4.27, p = 0.04 \), 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 male mating rank on fertilization success (all \( p > 0.25 \)): each male fertilized on average 21.3% of 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, but it dropped to zero during the third and fourth mating for UV-reduced males (Figure 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 female 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 behavior**

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. Laloi 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,
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which supports the hypothesis of trading-up mate choice in common lizards (Jennions & Petrie, 2000; Fitze et al., 2010; Laloi 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 shorter time with UV-control males than UV-reduced males. This result is counter-intuitive 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; unpublished results). 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 signals 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 have the advantage of providing higher sample sizes and allow 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, Bennett, & Cuthill, 2003; Zampiga, Gaibani, & Csermely, 2008; Leitão, Monteiro, & Mota, 2014), fishes (Kodric-Brown & Johnson, 2002; Macías Garcia & De Perera, 2002; Smith et al., 2002; Cummings, Rosenthal, & Ryan, 2003; Cummings et al., 2006; Boulcott, Walton, & Braithwaite, 2005; Rick, Modarressie, & Bakker, 2006), in one species of amphibians (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, Siefferman, & Hill, 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
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al. 2010). UV-based mate choice is perhaps more widespread than previously thought in lizards, and in vertebrates in general.

Furthermore, most experiments assessed female mate choice using simultaneous choice tests. These mate choice design consists in presenting simultaneously 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, Jennions, & Hunt, 2005). Yet, reproductive success of males is modulated by their ability to control the mating behavioral 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 behaviors 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 and thus overestimate their role. 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 the 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, but 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 (Appendix 2).

**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
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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 favor 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.

498 **Research data**

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

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**Figures legends**

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

**Figure 2.** Pairing success and duration in females according to the manipulation of the male UV throat coloration. The pairing success decreased in response to changes in trial order (from 1st to fourth behavioral 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 ± SE.

**Figure 3.** Proportion of fertilized eggs by males in 2012 and 2013 depending on their order of presentation to females and their UV treatment. Data are given as means (± 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.
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Figure 1

A. Number of bites versus trial order.

B. Number of bites for control and UV-reduced treatment groups.

C. Occurrence of female flips versus trial order for control and UV-reduced groups.
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Figure 2

![Graph A](image1)

![Graph B](image2)

Figure 3

![Graph 2012](image3)

![Graph 2013](image4)