Li *et al. J Biol Res-Thessalon (2023) 30:3*
https://doi.org/10.26262/jbrt.v30i0.9360

e-ISSN 2241-5793

LETTER TO THE EDITOR

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Is ultra-violet fluorescence a trait related to breeding in the Mongolian racerunner (*Eremias argus*; Lacertidae, Reptilia)?

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Abstract

Ornamental traits such as ultraviolet (UV) fluorescence and reflectance can provide reliable signals indicating the bearer's condition as a potential mate. UV fluorescence is widely found in nature and used for multiple functions, such as indicating female maturity in arachnids and acting as a signal enhancer in many insects. Lizards can display a broad range of colours, but the function of some of their ornamental traits remains unclear. Here, we report the presence of UV fluorescence in female *Eremias argus* specimens, a small lizard inhabiting Mongolia, China and the Korean Peninsula. Based on our observation, combined with the literature on the breeding behaviour of the species, we provide two hypotheses, assigning the use of UV fluorescence to: 1) an indicator of females' body conditions and 2) signal efficacy backup. To verify our hypotheses, we suggest a protocol with three serial trials under low light and enhanced UV light conditions.

Keywords: biofluorescence; lizard; mating; signal strengthening; true signal

Introduction

Numerous species rely on visually accessible traits for communication, such as reptiles, where some species with a vision spectrum considerably larger than that of humans also communicate through ultraviolet (UV) fluorescence and reflectance [1]. Fluorescence was first reported in reptiles in the 1970s, and most squamate species are expected to be able to see UV fluorescence and reflectance because of an ultraviolet-sensitive visual pigment, linked to four spectral classes of retinal cone cells. In addition, UV sensitive cones play an important role in UV perception in reptiles, as the short-wavelength-sensitive type 1 (SWS1) opsin is present in several lizard families, confirming tetrachromacy. As a result, the use of UV reflectance and induced fluorescence is widespread in reptiles; for instance, female Brazilian whiptail lizards (*Cnemidophorus ocellifer*) prefer males with high ultraviolet ornament reflectance [2].

While the function of fluorescence is understood in some reptile species, such as mate signalling in chameleons [1], it is still a question for others. For instance, specific body parts in some gecko species are fluorescent, with benefits potentially including mate selection, crypticity and predator avoidance. The strength of the UV re-

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flectance or induced fluorescence can also be an indicator of the body condition [3]. Fluorescence in lizards is, so far, known to be related to the fluorescence from bones, visible through the skin or specific transparent scales [1], and from the skin caused by iridophores [4]. Ultraviolet light can be divided into two ranges: the near UV spectral range (200-380 nm) and the far-UV spectral range (10-200 nm). So far, all reported cases of fluorescence in reptiles are within the near UV spectral range. This uniformity in results may, however, be linked to the commercial availability of black-lights resulting in UV fluorescence within this spectrum and further research including the far-UV spectral range is needed.

The signals linked to breeding in lizards can also rely on the use of fluorescence and reflectance. For instance, the strength of the UV colouration can also be an indicator of the body condition [3]. Generally, the mating behaviour includes precopulatory, copulatory and postcopulatory components (reviewed in [5]) as lizards have complex mating behaviours, including dewlap displays, head bobbing, push-ups and guarding. Male lizards will start displaying when they encounter a susceptible female, identifying her mating readiness through visual and chemical cues.

The Mongolia racerunner (*Eremias argus*) is a small lizard found in Mongolia, China and the Korean Peninsula. There is a notable sexual dimorphism, with males having orange/red flanks and abdomens and female lizards having light yellow/white abdomens [6]. Mating in *E. argus* can be divided into 12 sequential behaviours. Successful mating is initiated by the contact of the male's nostril with the female's cloacal region, followed by a bite on the female's trunk without releasing. After the first precopulatory bite, male inflicts copulatory bites on female's lower trunk region and then copulation follows. Finally, male inserts his genitalia into the female's cloaca from below (details in [7]).

Here, we provided hypotheses on the use of UV fluorescence in *E. argus* based on (1) the presence of UV fluorescence in museum specimens of 12 females, and (2) a review of the mating behaviours in the species. Finally, we provided protocols to test our hypotheses.

Hypotheses

Here, we provide two hypotheses related to the mating function of UV fluorescence on all claws of female *Eremias argus*. While energy allocation to sexual signals may be common in both female and male lizards, our hypotheses here are focused on females.

1. Female's condition indicator hypothesis

UV fluorescence in females is an ornamental trait, displaying their readiness for mating. As males of *E. argus* choose larger females [7], the signal is likely a unique trait in females that males can use to discriminate among mating partners (Figure 1A).

2. Efficacy backup hypothesis

We did not examine males and cannot confirm fluorescence on their claws. The fluorescence of claws in males, along with displaying in both sexes, would support the efficacy back-up hypothesis, where a trait is used to reinforce other signals, such as olfactory signals. In the case of social communication, species rely on complex colour patterns [8], especially when living in complex environments such as *E. argus* [9], are particularly likely to rely on fluorescence to reinforce communication signals. Therefore, the presence of UV fluorescence may be used to communicate between individuals, not only in the mating context (Figure 1B).

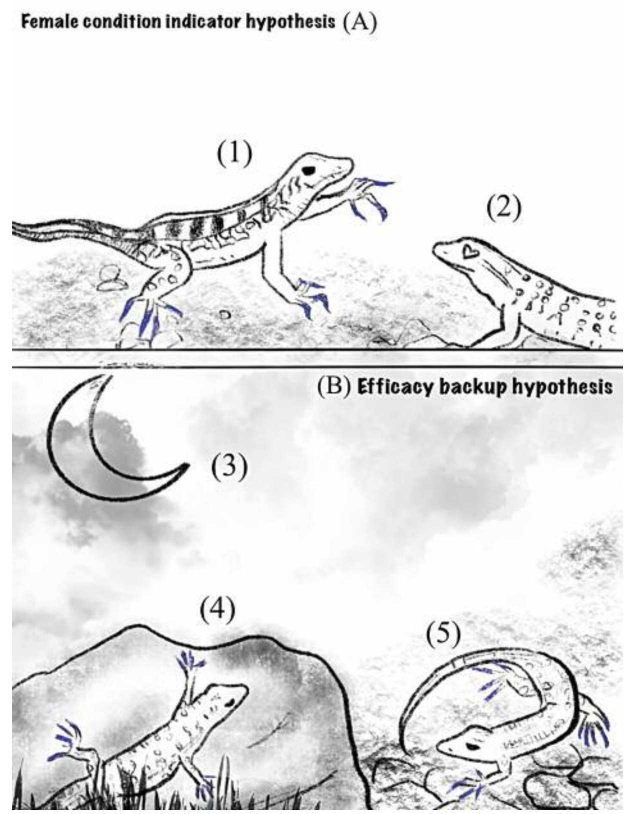

Figure 1. Illustration of the female condition indicator hypothesis (A) and efficacy backup hypothesis (B). (1) Display of true signals by females. (2) Detection and mate choice by males. (3) Increased light diffraction at dusk and dawn. (4) Visual signal strengthened in complex environments. (5) Intraspecific or interspecific observations.

Examination of museum vouchers

We examined 12 preserved vouchers of female *Eremias argus* deposited in the Ewha Womans University Natural History Museum, Republic of Korea (voucher series EWNHM-ANIMAL 6455-6577; [10]). The animals were collected between 1956 and 1959, across the northern regions of the country, and preserved in formaldehyde in temperature- and humidity-controlled conditions. It is unknown whether the preservative was changed during storage, but all vouchers were stored in transparent glass jars and the only matter in suspension was shed skin, for some individuals. Some specimens were however not preserved in full body extension and were folded over themselves. We used a black light (390-395 nm; Ultraviolet Blacklight Flashlight 51 LED, Escolite, Chicago, USA) through the glass of the storage jars to check for UV reflectance. We used a white fabric thread known to be reflecting fluorescence as control to ensure that the fluorescence observed was not an artefact. The thread was placed at the back of the jar during inspections. This protocol is reliable due to the multiple verifications, and comparable to doing the same tests on live individuals as the vouchers can be examined at length, and there is no ethical consideration.

All 12 individuals observed had claws reflecting or producing UV fluorescence under black light, for all claws on both front and back legs (Figure 2). The individuals were housed in four different jars, thus decreasing the risk of fluorescence due to fungi or reaction with the preservatives, and in contrast, the two *Takydromus* individuals housed in the same jar as some of the *E. argus* individuals were devoid of fluorescence. We tried to remove any potential fungi contamination from the claws of one of the *E. argus* specimens by rinsing the claws and scratching their surface softly, but the fluorescence persisted uniformly, highlighting the natural origin and not a fungal growth. In addition, the observation of the individuals under normal light conditions did not show any specific colouration that would result in perceived fluorescence (Figure 2).

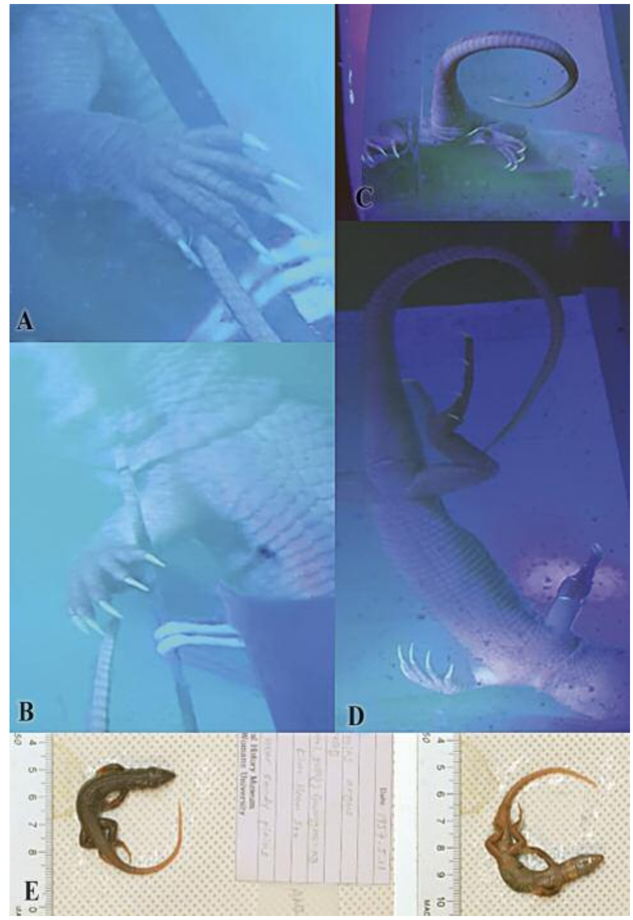

Figure 2. Details for fluorescence under UV-light in *Eremias argus*. All 12 specimens of *Eremias argus* preserved as vouchers (ID EWNHM-ANIMAL 6455-6577) at the Ewha Womans University Natural History Museum, Republic of Korea tested positive for fluorescence on all nails. (A-D) Fluorescence under UV-light on the nails of *E. argus*. (E) Dorsal and ventral observation of the voucher under normal light.

Support to the hypotheses

Ornamental traits are widely understood to be reliable indicators of individual condition and quality. The cost of sexual signals drives the evolution of condition-dependence as the only individuals able to pay the price of exaggerated signals are the ones with higher body conditions [11]. In female striped plateau lizards (*Sceloporus virgatus*), components of skin lipids correlate with clutch size and the chroma of a female's orange throat patch is also related to the clutch size [12].

Many species use redundancy in signals to convey the message adequately and increase the chance of mating. Here, UV fluorescence is a "backup signal", where it reinforces a visual or olfactory message. Similarly, female European tree frogs (*Hyla arborea*) rely on visual signals to discriminate between males with equal call properties [13] and fluorescence regularly enhances colour signals [14].

Communication in complex environments is difficult and animals have evolved multimodal signalling to adapt to this environment. Each signal can highlight female reproductivity status, but all the signals displayed have a synergistic effect [15]. *Eremias argus* is therefore likely to use UV fluorescence as a backup signal in courtship. The species lives in complex environments such as dunes with 75% plant coverage and medium-grained sand [9], and hence it is likely that it relies on this trait under low-light environments to discriminate potential mates and assess their body condition when conspicuous colours become less noticeable but the light environment shifts to shorter wavelengths and UV reflectance increases, such as at dawn and dusk. A similar time-dependent behaviour is used by the Woodland brown butterfly (*Lopinga achine*), which recognizes the deflective role of UV reflecting patterns increased at dawn.

Hypotheses verification

To verify our hypotheses, we recommend the following protocol, relying on a sufficient number of replicates to conduct appropriate statistical analyses. (1) The first trial is a control observation to establish a baseline against which the results of the manipulative experiments can be compared. Each of the subsequent tests is a manipulative experiment consisting of a mate preference test, with males selecting females. The experiments are conducted in laboratory conditions at 25 - 26 °C under low light condition and black light to mimic dawn and dusk conditions during the mating season, and enhance the fluorescence traits. Each individual is measured, weighed and colour measurements are taken to be used as covariates. All trials are conducted in a testing arena measuring 100 cm length × 100 cm width × 60 cm high transparent walls with camera recording from above and each of the sides for further analyses. This first control trial consists of males freely selecting females without any alterations. In the first trial, the claws of all females are covered with a UV absorbent get (such a used for humans in follow up of injuries or surgery; e.g. KELO-COTE®, Alliance Pharma, UK) to hide the fluorescence. The follow-up manipulative trials are conducted under the same conditions, with the following specificities. (2) In the second trial, the fluorescence levels for all females are enhanced by utilizing removeable UV painting pens (e.g. Edding, Germany) on their nails to reach a not significantly different level. (3) In the third trial, we hinder the fluorescence-related attractive traits of the most "successful" individuals such as done in Trial 1, and enhance the fluorescence-related trait of the least "successful" individuals with the removeable UV painting pen. To demonstrate the use of fluorescence for mate selection, we expect the mate choices of trials 2 and 3 to be random compared to trial 1, and the results of the third trial to be opposite to that of the control (Figure 3).

We also suggest to consider the following recommendation to select individuals for the experiments. The adult lizards should be selected according to their age, ensuring that all individuals are sexually mature. This includes considerations such as emergence from hibernation and mating season. Males should be selected to have similar snout-vent-length (SVL) to reflect a similar experience and similar mating coloration, specifically on their throats to avoid influencing the behaviour of females. In opposition, females should be selected within a specific range of SVL (measured and used as random factors in the statistical analyses) to generate the variations needed for the two manipulative trials.

Conclusion

Fluorescence can be structural, for instance on the wings of butterflies. UV reflectance in butterflies is created through interference by lamellae on wing ridges, such as in the Orange sulphur butterfly (*Colias eurytheme*) and the Common egg fly (*Hypolimnas bolina*). In addition, fluorescence can be chemical, and for instance, the swallow tail (*Papilio nireus*) emits fluorescence through scales consisting of hollow air cylinders on its wings, where the fluorescent pigment is located. Similarly, most insects are usually covered by a cuticle that can produce a fluorescent compound derived from pterins and dragonflies possess a fluorescent protein structure in their wings, emitting blue lights facilitating intraspecific recognition. Hence, the chemical or structural origin of fluorescence in *E. argus* needs to be determined as it could be the result of either process. Further studies such as testing behaviours on different substrates or interference of other signals to evaluate the functions of this unique trait need to be conducted.

Limitations to the protocol suggested here to determine the use of UV fluorescent cues in social interactions is that the use of the far-UV spectral range (10-200 nm) would not be detected. It is however unlikely to be used as the rod structure of squamate for which data is available does not match this range of wavelengths. In addition, ontogenic factors and the maintenance of signals may not be the only energetic costs. For instance, variations in the hormonal balance, immune system, oxidative balance and parasite load can also affect the health of individuals, and thus also affecting signals.

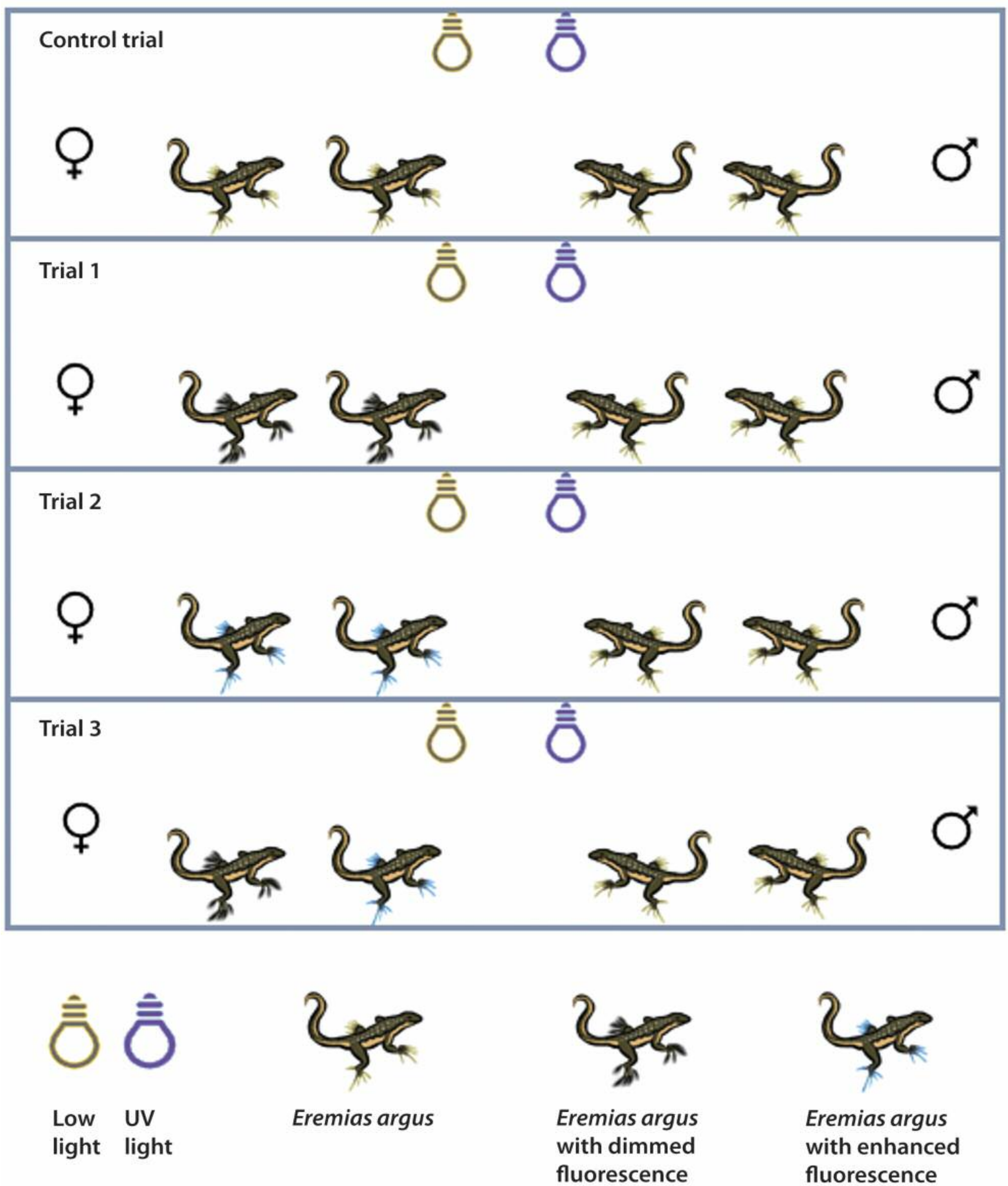

Figure 3. Protocol to test the hypotheses, composed of a control observation and three manipulative experiments relying on different mate conditions. The experiments are conducted in laboratory conditions under low light and UV light. The control trial consists of males freely selecting females without any alterations. In the first trial, the claws of all females are covered to hide the fluorescence. In the second trial, the fluorescence levels for all females are increased to reach a shared level. In the third trial, we hinder the fluorescence-related attractive traits of the most "successful" individuals, and enhance the fluorescence-related trait of the least "successful" individuals.

Acknowledgments
We are grateful to the reviewers for spending time reviewing our work.

Declarations
This project is based on museum specimens; therefore, no research ethics approval is applicable.

Availability of data
All data is presented in the manuscript.

Competing Interests
The authors declare not to have competing interests.

Funding
This work was supported by the Foreign Youth Talent Program (QN2021014013L) from the Ministry of Science and Technology to AB; and partially funded by the Hainan Natural Science Foundation (320MS039), Hainan Students Innovation and Entrepreneurship Training Program (S202111658021) and the National Natural Science Foundation of China (31870506).

Authors' contributions
Conceptualization: JL, AB; Formal analysis: JL, AB; Funding acquisition: AB, MH, YJ; Visualization: JL, AB; Writing – original draft: JL, AB; Writing – review & editing: JL, AB, YS, MH, YJ.

Received: 20 March 2023
Accepted: 28 March 2023
Published: 4 May 2023

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