Introduction

The study of animal communication is a complex science addressing a wide range of multi-layered questions, such as how a signal is emitted (e.g., visually, acoustically etc), how it is perceived (e.g. the spectral range and sensitivity of colour vision), and of course the adaptive reasons for signaling (e.g., deterring rivals, attracting mates). Any and all of these factors interact to mold the adaptive reasons for signaling (e.g., deterring rivals, female choice). The role of UV in communication has, however, never been examined.

Background: Recent work on animal signals has revealed a wide occurrence of UV signals in tetrapods, in particular birds, but also in lizards (and perhaps other Squamate reptiles). Our previous work on the Swedish sand lizard (Lacerta agilis) has verified, both in correlative selection analyses in the wild and with laboratory and field experiments, the importance of the green ‘badge’ on the body sides of adult males for securing mating opportunities, probably mostly through deterring rival males rather than attracting females. The role of UV in communication has, however, never been examined.

Methodology/Principal Findings: Here we show that when measured immediately after spring skin shedding, there is also signaling in the UV. By UV-depriving the signal (reflectance) with sun block chemicals fixated with permeable, harmless spray dressing, we show that males in the control group (spray dressing only) had significantly higher success in mate acquisition than UV-deprived males.

Conclusions/Significance: These results suggest that at least two colour traits in sand lizards, badge area and UV, contribute to rival deterrence and/or female choice on UV characters, which elevates success in mate acquisition in UV intact male sand lizards.
Materials and Methods

The field work in this population (Asketunneln, Sweden 57º22’ E11º58’) follows a well-established protocol that has been reported on in previous work (e.g., [9–12,22]). In short, sand lizards (*Lacerta agilis*) are small (to 20 g), ground-dwelling lizards. Eighty five males and eighty females are individually marked short term by putting a uniquely numbered cloth tape on their backs. Males observed courting, copulating or mate guarding females were classified as partners. The sex ratio in the Asketunneln population is approximately 1:1, the capture rate of adults is >90% and, thus, the observations made of the lizards in the current paper are based on nearly complete coverage of the adult population. That said, scored mating success of adult males is known to covary with the number of times males are observed, which was therefore controlled for in our analyses. All adults were weighed (to the nearest 0.1 g), measured (snout-vent and total length to the nearest 1 mm), and a 50 ml blood sample was taken from vena angularis (in the corner of the mouth) of both males and females and stored in 70% alcohol for later molecular genetic analysis. Males and females were then released at the place of capture and monitored during the mating season (ca seven weeks) every day that weather permitted.

Females were immediately released at the place of captured. Males were accumulated at daily field captures over a ten day period and stored at +8°C in a constant temperature room, awaiting a synchronized release of all males immediately after being weighed, measured, marked and treated with UV blocker (released 2 May 2008). Representative radiospectrometric analysis of UV blockage effects were performed at release and after three weeks (Fig. 1) to verify that our UV blockage had the desired long-term effects. The second measure after three weeks was virtually indistinguishable from the first (Fig. 1). The UV blockage was performed by gently rubbing +50 SPF (‘sun protection factor’; Vichy Laboratoires, Capital Soleil, Very High Protection) on every second male (*n* = 43, for controls, *n* = 42) in an Excel size-sorted data set of the captured males in storage. This ensured that UV-blocked and control males did not differ in snout-vent length, mass or body condition (*p* > 0.14 for all three of these traits).

Figure 1. UV/VIS spectral reflectance from sand lizard flanks: wild (long-short dash), control-manipulated (solid lines), UV-manipulated 1 hour (dash), and 30 days (points) after treatment. Note the considerable UV-reduction also after 30 days in the wild. Spectra are set to equal brightness, in order to see spectral shape (i.e., colour) more precisely.

doi:10.1371/journal.pone.0019360.g001

After the morphology data had been collected and the lizards treated, they were released at random places of capture (i.e., randomizing sites that were, at capture, potentially further or closer from females) and monitored for associations with partners (facilitated by the prolonged mate guarding, [22]) every day of the mating season when the weather permitted lizard activity (3 May–20 June, number of observation days, *n* = 26). Thus, our procedure also eliminates variation in male spring emergence (since all males are released simultaneously). Our work was approved by the Animal Ethics Committee, University of Gothenburg.

Our statistical analyses involved two approaches: (a) we first performed a homogeneity of slopes regression analysis with number of partners as response variable and treatment (UV-blocked vs. controls), number of observations of a male and its interaction with treatment, and male snout-vent length as covariate. However, because of some non-normality of the data (over-representation of zero pairing success), we (b) also performed an analysis more robust to deviation from normality using a logistic regression with an ordered cumulative logit model with the same trait variables.

Results

There was no difference in the mean number of observations of UV-reduced and control males (mean number of re-observations, 2.1 ± 0.24, range 1 to 8, and 1.93 ± 0.24, range 1 to 9, for control and UV-reduced males, respectively; *T*-test, *t* = 0.61, *P* = 0.54). Across treatment and control males, the number of observations of
a male after release was correlated with the number of times he was seen courting a female ($r_s = 0.49, P < 0.0001, N = 85$). We therefore incorporated male number of re-sightings in our analysis of treatment effects on number of females paired. UV-blocked males had an average of 0.12 female pairing observations per male ($\pm 0.049$, SE, $N = 43$), whereas the corresponding number for control males was three times as high ($0.31 \pm 0.12, N = 42$). The regression analysis was globally significant ($F_{6, 81} = 40.7, P < 0.0001, R^2 = 0.60$), and had significant independent effects of treatment ($F = 15.4, P < 0.0002, d.f. = 1$), number of observations ($F = 92.9, P < 0.0001, d.f. = 1$), and their interaction ($F = 39.7, P < 0.0001$; Fig. 2). Body size (SVL) was backwards eliminated from the final model ($P > 0.25$). Our cumulative, ordered logistic regression largely agreed with these results (Global model Likelihood ratio $X^2 = 15.4, P < 0.0002, d.f. = 1$), number of observations ($X^2 = 35.0, P < 0.0001, d.f. = 4$). The number of observations of a male significantly affected the number of females he was observed with ($Wald X^2 = 15.06, P = 0.0001$), the treatment x observation interaction remained significant ($Wald X^2 = 6.03, P = 0.014$), while the treatment effect per se fell just short of significant ($Wald X^2 = 3.07, P = 0.079$; Fig. 2).

Discussion

Our results show slight discrepancy between the logistic regression and the linear multiple regression analysis. However, we know from previous work [22] that the number of observations per male influences estimates of mate acquisition (number of females seen courting), and that this effect is modified by UV reduced signaling. Thus, it can be argued that the significant interaction term in both analyses is the correct unit of analysis, and it is significant in both cases. How robust are these results? The current study is specifically aimed at analyzing mate acquisition success in relation to UV blockage. Thus, analyzing access to females is a more appropriate level of analysis than tallying reduction (grey) versus control males (black).

Our cumulative, ordered logistic regression largely agreed with these results (Global model Likelihood ratio $X^2 = 15.4, P < 0.0002, d.f. = 1$), number of observations ($X^2 = 35.0, P < 0.0001, d.f. = 4$). The number of observations of a male significantly affected the number of females he was observed with ($Wald X^2 = 15.06, P = 0.0001$), the treatment x observation interaction remained significant ($Wald X^2 = 6.03, P = 0.014$), while the treatment effect per se fell just short of significant ($Wald X^2 = 3.07, P = 0.079$; Fig. 2).

**Figure 2.** Mating success in male sand lizards depending on UV reduction (grey) versus control males (black). Increment symbol size represents increasing number of observations of males from 1 (smallest) to 24 (largest).

doi:10.1371/journal.pone.0019360.g002

Our results and interpretations also agree with those of two previous studies on the role of UV in lizard communication. Stapley and Whiting [25] showed with a field experiment that males with reduced UV signals in *Platysaurus broadleyi* were more likely to be challenged by rivals, and Bajer et al. [26] showed that male green lizards (*Lacerta viridis*) with reduced UV signals were less spatially associated with by females. In sand lizards, we have never been able to demonstrate that there is female choice on male colour traits whereas there are strong effects of male green badges on male contest behaviours [9,10]. Thus, we conclude that male UV reduction in this species compromises mate acquisition but that it is unresolved in free-ranging animals whether this is a combined effect of male-male rivalry and female choice on UV components of signalling.

In summary, our field experiment demonstrates a technique for long-term elimination of UV signaling in free-ranging lizards, which reduces success in mate acquisition, probably through reduced deterrence of rivals.

**Author Contributions**

Conceived and designed the experiments: MO SA. Performed the experiments: MO EW. Analyzed the data: MO. Contributed reagents/materials/analysis tools: MO SA. Wrote the paper: MO.

**References**

1. Andersson M (1986) Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution 40: 804–816.

2. Grafen A (1990a) Sexual selection unhandicapped by the Fisher process. J Theor Biol 144: 473–516.

3. Grafen A (1990b) Biological signals as handicaps. J Theo Biol 144: 517–546.

4. Hasson O (1997) Towards a general theory of biological signaling. J Theor Biol 185: 139–156.

5. Rohwer S (1976) The evolution of reliable and unreliable badges of fighting ability. Ann Zool 22: 531–546.

6. Zahavi A (1977) The cost of honesty (Further remarks on the handicap principle). J Theor Biol 67: 605–605.

7. Searcy WA, Nowicki S (2005) The evolution of animal signaling systems. Monographs in Behavior and Ecology Princeton: Princeton University Press.

8. Fryke S, Andersson S (2003) Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. Anim Behav 66: 217–224.

9. Olsson M (1994a) Nuptial coloration in the sand lizard (*Lacerta agilis*): an intrasexually selected cue to fighting ability. Anim Behav 46: 607–613.

10. Olsson M (1994b) Rival recognition affects male contest behavior in sand lizards *Lacerta agilis*. Behav Ecol Sociobiol 35: 249–252.

11. Olsson M (1994c) Why are sand lizard *Lacerta agilis* males not equally green? Behav Ecol Sociobiol 35: 169–173.
12. Anderholm S, Olsson M, Wapstra E, Ryberg K (2004) Fit and fat from enlarged badges. Biol Lett 271: 142–144.
13. Toveé MJ (1995) Ultra-violet photoreceptors in the animal kingdom: their distribution and function. Trends Ecol Evol 10: 453–460.
14. Andersson S, Amundsen T (1997) Ultraviolet colour vision and ornamentation in blue throats. Proc Roy Soc Lond B 264: 1587–1591.
15. Bennett ATD, Cuthill IC, Partridge JC, Lunau K (1997) Ultraviolet plumage colors predict mate preferences in starlings. Proc Natl Acad Sci USA 94: 9618–9621.
16. Delhey K, Johnse A, Peters A, Andersson S, Kampenaers B (2003) Paternity analysis opposing selection on crown coloration in the blue tit (Parus caeruleus). Proc Roy Soc Lond B 270: 2037–2063.
17. Lopey Jr. GS (2003) Crypsis and communication functions of UV-visible coloration in two coral reef damselfish Dascyllus aruanus and D reticulates. Anim Behav 66: 299–307.
18. Li D, Lam LM (2004) Ultraviolet cues affect the foraging behaviour of jumping spiders. Anim Behav 70: 771–776.
19. De Lanuza GPI, Font E (2007) Ultraviolet reflectance of nuptial coloration in sand lizards (Lacerta agilis) from the Pyrenees. Amphib Reptil 28: 438–443.
20. Martin J, Lopez P (2009) Multiple color signals may reveal multiple messages in male Schreiber’s green lizard Lacerta schreiberi. Behav Ecol Sociobiol 63: 1743–1755.
21. Whiting MJ, Stuart-Fox DM, O'Connor D, Firth D, Bennett NC, et al. (2006) Ultraviolet signals ultra-aggression in a lizard. Anim Behav 72: 353–363.
22. Olsson M, Wapstra E, Madsen T, Silverin B (2000) Testosterone Ticks and Travele: a test of the immunocompetence-handicap hypothesis in free-ranging lizards. Proc Roy Soc B 267: 1–5.
23. Olsson M, Shine R, Madsen T, Gullberg A, Tegelström H (1996) Sperm selection by females. Nature 383: 583.
24. Gullberg A, Olsson M, Tegelström H (1997) Male mating success reproductive success and multiple paternity in a natural population of sand lizards: behavioural and molecular genetics data. Mol Ecol 6: 105–112.
25. Stapley J, Whiting MJ (2006) Ultraviolet signals fighting ability in a lizard. Biol Lett 2: 169–172.
26. Barja K, Molnár O, Tórók J, Heczeg G (2010) Female European green lizards (Lacerta viridis) prefer males with high ultraviolet throat reflectance. Behav Ecol Sociobiol 64: 2007–2014.