Theses of the dissertation

UV-COLOURATION: SEXUAL SELECTION AND MATERNAL INVESTMENT IN EUROPEAN GREEN LIZARD (*LACERTA VIRIDIS*)

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1 Introduction and aims

Visual, acoustic and chemical signals and behavioural displays that are advantageous in intra- and/or intersexual selection can play a role as a signal of phenotypic condition, social or reproductive status during signalization (Andersson 1994).

Based on these secondary sexual traits, individuals of the same sex can assess the probable outcome of the contest with their rival, and are able to avoid the energetically costly physical fight, thus decrease predation risk (Rohwer 1975). By assessing these traits females gather information about direct (territory quality, feeding rate) and indirect (genetic quality of the male) benefits gained by mating with the bearer of the trait (Andersson 1994).

Sexual signals can honestly advertise individual quality through their producing and maintenance cost, and only individuals of better quality can afford intensive elaboration of those signals (Zahavi 1975, Grafen 1990). Pigment based and structural colours are synthetized through various chemical pathways with different costs, therefore they are able to signal different aspects of individual quality. Structural colours do not need pigment molecules, therefore they were regarded as not energetically costly to produce. (Prum & Torres 2004). Recent studies showed that production and maintenance of structural can be as costly as pigment based colours (Kemp 2008).

There is a trade-off between present and future reproduction and survival of the individuals, as every reproduction has a cost that decreases the probability of future reproduction and survival of the individual (Williams 1966). Therefore females have to optimize their reproductive investment according to the quality and prospective costs of progeny, and the prospective quality of progeny arising from future matings (Trivers 1972). The benefits of mating with an attractive male and the rate of their heritability can influence offspring quality that leads to differential allocation according to male quality. Positive differential allocation (Burley 1988) strategy means that females would invest more heavily into clutches sired by preferred males. Compensation strategy (Gowaty 2008) is expected to be present if females do not have to opportunity to make their choice between males and they have to mate with a non-preferred male. In this case it would be worth for females to invest more into clutches sired by unattractive male, compensating for worse offspring quality.

In my dissertation, I investigated the role of the UV colouration in forming the patterns of reproduction and maternal investment from four different aspects in European green lizard. I used manipulative experiments to test the potential role of the signal in female mate preference and male contest. Then, I used another experiment to investigate the effect of environmental / energetic constraints on UV colouration. Last, I tested the reproductive allocation of females according to the males' UV colouration as a sexually selected trait.

1.1 Mate preference

I used manipulative experiments to reveal if European green lizard females make a choice between males based on difference in their UV colouration expressed during reproductive season. In order to test the role of UV colour independently of other unknown traits possibly correlating with UV, I manipulated the UV reflectance of males' throats. I showed females males differing systematically only in UV colour, but not in any other morphological characters. My aim was to test the hypothesis that UV colouration might be a base for female preference and that the UV colour expression might predict patterns of mate preference.

2. Male contest

I tested the effect of artificially induced difference in UV colouration of males not differing systematically in any other morphological characters in staged encounters tests, where I identified winners and losers during the contest competition of males with manipulated nuptial signal. I tried to reveal if the difference in UV colour does predict the outcome of male contest. By investigating the same trait in both intra- and intersexual selection, I tried to implicate to the evolutionary process acting on forming this potential signal.

3. Colour development

Cost of UV colours are poorly understood, there are no previous studies investigating ectotherm vertebrates. I investigated the effect of two fundamental environmental factors (food and temperature) on the annual development of UV colouration. I tried to reveal if the structural colour expression of *L. viridis* is influenced by (i) food, (ii) the time individuals were able to achieve high body temperature or (iii) the interaction of these two factors?

4. Maternal investment

In this experiment I manipulated the natural sexual traits of males. I showed males of different quality (within a pair) to females and identified preferred and non-preferred males. In the next stage of the experiment, I let the half of the females mate with the preferred male, the rest were restricted to mate with non-preferred males. I compared the attributes of clutches sired by males of different preference-status. I aimed to reveal if there is a difference between the maternal investments (in terms of clutch size and egg weight) of clutches sired by preferred and non-preferred males?

2 Methods

2.1 Study species

The European green lizard is the largest lizard species in Hungary (SVL = 80–120 mm) and it is widely distributed in our country. Males have a bright blue nuptial colour with considerable UV reflectance on their throat in the reproductive season (pl. Václav *et al*. 2007).

2.2 General methods

Right after hibernation males' throat is pale grey and it progressively turns to be bright blue, reaching its intensity peak during the mating season. After that it starts to fade out in July and disappears completely by the end of September. Despite that there is only limited data available on annual dynamics of UV colouration of males, these suggest that the UV component of the throat patch might undergo the same development as blue – the other (structural) component of the throat patch.

I conducted the experiments in April-May of 2007, 2009 and 2011. I collected the individuals in every year within six days in order to minimalize the time differences between individuals spent in captivity. I made sure that the females were unmated by checking them for mating scars. Individuals in colour development experiment were collected within four days, in order to make sure that the time spent in captivity before the experiments (*ad libitum food*) will not create differences in the initial colouration between the different treatment groups.

2.3 Experiments

2.3.1 Mate preference

I manipulated the UV component of the nuptial colouration of males, and tested the female preference showed toward the manipulated trait. I assembled 20 male pairs with the members differing in their SVL at a maximum of 2 mm. In order to be able to test the role of the manipulated trait in mate preference independently of other unknown traits, I manipulated the UV reflectance of the members of a male pair. I designated males as UV reduced or control randomly, and treated controls only with wax, while UV reduced males were treated with a UV reducing agent, as well. I used a special arena for the behavioural tests, in which the female could only see, but not sense the males' chemical cues and assumed they stay in the near of the preferred male (LeBas & Marshall 2000). I recorded the position of the female in every ten minutes for eight hours.

2.3.2 Male contest

I assembled male pairs with assumptions and methods identical to those in mate preference tests. Experiments were conducted in special arenas that could be divided into two. I placed males in their own compartment and let them rest for ten minutes to acclimatize. After that, I pulled the bulkhead and monitored the aggressive interaction (approaching the rival male with head lowered, throat inflated, back arched) of males in order to identify winners and losers.

2.3.3 Colour development

I investigated the effect of two treatments on colour development. In "high food" treatment males received 10 ml *Tenebrio molitor* larvae three times a day (*ad libitum* feeding). In "low food treatment" males received 2 ml larvae three times a day. I used two different temperature treatments. In "high temperature" treatment the heater was on for ten hours a day, but in "low temperature" treatment only for five hours a day. I measured the bodyweight and reflectance spectra twice, once before the experiments started (initial) and once right after the experiments ended (final).

2.3.4 Maternal investment

I tested 35 females in the mate preference identical to those above in the 2.3.1 *Mate preference* section. After identifying preferred and non-preferred males, I let half of the females mate with their preferred male (N=32), the other half of the females was restricted to mate with the non-preferred male. After the mating scars appeared on females, I took the males out and kept females in terraria until they laid their eggs. I recorded clutch size and egg weight for every female.

2.4 Statistical methods

I used paired t-tests when testing for the difference in morphology or colouration between males of different treatment groups (UV-reduced, control) before the treatments in the experiments of mate preference, male contest and maternal investment. I repeated the test after the treatments, and also used paired t-test for testing the female mate preference. Mate preference was also re-tested using Wilcoxon matched-pairs test. I used GLMs to see if the UV reduction was successful in the mate preference, male contest and maternal investment experiments, and also for testing the effect of preference status and parental attributes on clutch size in the maternal investment experiment. I also used GLMs to see if there was an effect of the treatments on UV development in colour development experiments. GLMMs were used to test the effects of parental attributes, male preference status and clutch size on the egg weight in the maternal investment experiment.

3 Results and discussion

3.1 Mate preference

My results show that UV colouration plays a role in both mate preference and male contest. During mate preference, receptive but unmated females stayed often in the near of males bearing more intensive UV throat patch. Intensive UV reflectance can signal (1) male quality (not only genetic quality, but health status) or (2) quality of the territory.

Evidences for costs of structural colour traits were found only recently, for example it can be condition-dependent and there might be considerable costs linked to its development or maintenance (Olsson 1993). Moreover, it might be affected by parasite load (Molnár *et al.* 2012). These results suggest that structural throat colouration can be an honest signal of health status.

3.2 Male contest

Males with reduced UV reflectance were more likely to lose contests than control males. Manipulated UV colouration predicted the outcome of male contest with a probability of 88 %. These results suggest that the UV reflectance of the throat patch can be a reliable signal during male contest in *L. viridis*. By assessing status signals, males are able to gather information about the dominance or aggression of their rival. This way they can avoid energetically costly fighting and decrease the risk of injuries and predation (López *et al.*

2004), saving energy that can be allocated into feeding, mating or thermoregulation. (Whiting *et al.* 2003). Throat colouration can work as an amplifier, fostering the assessment of head size that often correlates with bite force (Lappin *et al.* 2006). The fact that UV colour plays a role in both contexts (intra- and intersexual selection) rejects the assumption that UV throat patch was developed by Fisherian runaway selection.

3.3 Colour development

The annual development of the structural colour of *L. viridis* was influenced by the time during which high body temperature was available for in males. Interestingly, food treatment that affected body condition did not affect UV colour expression. Moreover, the time available to achieve high body temperature that affected the rate of UV reflectance growth, had no effect on body condition. Quality of structural colours depends on light reflecting and light scattering ultrastructures of the epidermis. Environmental stress can disturb the biochemical pathways during which ultrastructures are produced, therefore these stress factors can influence the development of structural colours (Kemp 2008). Developmental stability is temperature dependent in ectotherms (Imasheva *et al.* 1997), thus the suboptimal temperature can cause a less intensive seasonal colour development even in adult individuals.

3. 4 Maternal investment

My results support the positive differential allocation hypothesis (Burley 1988). Females showed strong preference toward males with more intensive UV reflectance and invested differently into clutches sired by preferred and non-preferred males, resulting in higher investment (in terms of egg weight) into clutches sired by preferred males. Egg weight was also affected by clutch size, showing the limited energy that can be allocated into reproduction (Williams 2001). Moreover, females choosing from males of bigger mean body size laid more eggs, independently of preference status of the male. Heavier eggs can increase offspring quality through various ways: offsprings hatching from bigger eggs show better survival and higher growth rate (Krist 2011). Using this experiment design, I was able to reproduce a male structural colour trait and the maternal investment related to it, independently of other unknown, untested effects. In other words, in this case female preference showed toward the male UV colour signal could be caused by the differential allocation showed by females *per see*.

Result new to the scietific field of behavioural ecology:

(i) This is the first study to find female mate preference for a structural colour signal (and colour signal in general) in reptiles.

(ii) I showed the importance of UV colour during sexual selection independently of other traits potentially effecting preference.

(iii) I showed the role of UV colour in male contest of European green lizard.

(iv) I found evidence for the limitating effect of temperature during the signalization of an ectoterm vertebrate.

(v) I showed relationship between a fundamental manifestation of maternal investment (egg weight) and the preference status of the sire.

(vi) By manipulating a natural sexual trait, I found evidence for positive differential allocation in a reptile.

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4 Publications and manuscripts

4.1 Publications and manuscripts included in the thesis

Bajer K, Molnár OR, Török J, Herczeg G (2010) Female European green lizards (Lacerta viridis) prefer males with high ultraviolet throat reflectance. Behav Ecol Sociobiol 64, 2007–2014.

Bajer K, Molnár OR, Török J, Herczeg G (2011) Ultraviolet nuptial colour determines fight success in male European green lizards (*Lacerta viridis*). Biol Lett 7, 866-868.

Bajer K, Molnár O, Török J, Herczeg G (2012) Temperature, but not available energy, affects the expression of a sexually selected ultraviolet (UV) colour trait in male European green Lizards. PLoS ONE 7(3): e34359.

Bajer K, Molnár O, Török J, Herczeg G Male attractiveness and differential maternal investment in a lizard with sexually selected UV colour (manuscript)

4.2 Other publications

Molnár O, **Bajer K,** Herczeg G, Török J (2012) Individual quality and nuptial throat colour in male European green lizards *J Zool* 287, 233–239.

Bajer K, Molnár O, Hegyi G, Herczeg G, Laczi M, Török J (2010) Zöld gyíkok színezete és morfológiája: jelzések és funkciók. *Állattani Közlemények,* 94, 11-23.

Ihász N, **Bajer K**, Kopena R, Molnár O, Herczeg G, Török J (2006) Szemben a ragadozóval- a zöld gyík (*Lacerta viridis*) búvóhelyközpontú menekülési stratégiája *Állattani Közlemények* 91, 127-138.