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Melanism in common lizards (Squamata: Lacertidae: *Zootoca vivipara*): new evidence for a rare but widespread ancestral polymorphism

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Abstract. The presence of a dark-coloured body colouration polymorphism (melanism) is a pervasive phenomenon in the animal kingdom, particularly in reptiles. We provide the first reporting of melanic individuals in a subspecies of common lizards, *Zootoca vivipara carniolica* or the Eastern oviparous lineage. Two melanic females were found out of 194 individuals collected. Melanic females did not differ in size or weight from non-melanic females. No melanic individuals were found (N = 134) in the nearby viviparous population. Melanism has been reported in related lineages of *Z. vivipara*, so the discovery in this sister to all other lineages suggests that it is an ancestral polymorphism. The frequency of melanism varies but other studies also find it is usually very rare (<3%) and may be sex-biased. The processes mediating advantages and disadvantages of melanism in *Z. vivipara* are unclear and require more research.

Keywords. Oviparous, viviparous, adaptation, rare phenotype, altitude, camouflage, thermoregulation

Introduction

Body colourations and associated patterns vary between species and individuals in the animal kingdom. Colour polymorphisms within species are found in a number of amphibians and reptiles and may for example relate to camouflage in spatio-temporally diverse habitats, courtship signalling between males and females, thermoregulation, or dominance (Stuart-Fox et al., 2004; Kusche, Elmer and Meyer, 2015). Darkly pigmented (melanic) varieties within species are a relatively common colour polymorphism in vertebrates (Kronforst et al., 2012). Melanic phenotypes (morphs) are proposed to have a greater frequency in higher latitudes and altitudes, as shown for example on frogs (Alho et al., 2010), snakes (e.g. Luiselli 1992; Monney, Luiselli and Capula, 1995), and lizards (Clusella-Trullas, van Wyk and Spotila, 2007; Clusella-Trullas, van Wyk and Spotila, 2009). Experimental research has shown that melanic ectotherms heat up more quickly than non-melanic individuals (Gibson and Falls, 1979; Jong, Gussekloo and Brakefield, 1996). This has led to the hypothesis that selection for melanism in colder climates is stronger due to enhanced thermoregulation abilities in ectothermic animals, where dark pigments absorbs solar energy at a greater speed compared to light colours (Bonato and Steinfartz 2005; Clusella-Trullas, van Wyk and Spotila, 2007; Clusella-Trullas et al., 2008).

The common lizard (*Zootoca vivipara*) is a broadly distributed species across Europe and Asia (Surget-Groba et al., 2006), with currently six major lineages recognised (eastern oviparous [*Zootoca vivipara carniolica*], eastern viviparous, western oviparous [*Zootoca vivipara louislantzi*], western viviparous, central viviparous I, central viviparous II) (Table 1). *Zootoca vivipara* is a cold-adapted species, with the most northerly distribution of any lizard, nearing the Arctic Circle, and occurrence at altitudes of up to 2400 m a.s.l. (Schmidtler and Böhme, 2011; Rodríguez-Díaz and Braña, 2012). The presence of occasional melanic individuals in *Z. vivipara* has been noted since the early 19th century (Schmidtler and Böhme, 2011). In recent years however, there have been several sightings of melanic *Z. vivipara*, which have been described

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Table 1. Origin, sex, frequency, mean size (snout vent length, SVL) and weight of normal and melanic morphs in the oviparous (*Z. v. carniolica*; Eastern oviparous lineage) and the viviparous morph (Central viviparous II) at the sample location and compared to values from the literature of different lineages. Standard deviations (+/-) are shown in brackets. Body condition is measured here as weight/SVL ratio to allow comparison across studies.

lineage	region	sex	morph	N	SVL	weight	TL	body condition	melanism frequency
Eastern oviparous *Z. v. carniolica*[1]	Gailtal, Austria	female	normal	97	58.1 (4.7)	4.8 (1.2)	84.7 (12.6)	0.081	0.0202
			melanic	2	50.3 (5.6)	3.2 (1.1)	89.2 (24.9)	0.063	
		male	normal	95	51.6 (3.4)	3.7 (0.6)	84.3 (11.3)	0.071	0
			melanic	0	-	-	-	-	
Central viviparous II[1]	Gailtal, Austria	female	normal	89	63.2 (5.5)	5.8 (1.7)	83.3 (11.0)	0.091	0
			melanic	0	-	-	-	-	
		male	normal	45	50.1 (3.1)	3.6 (0.8)	85.8 (12.2)	0.070	0
			melanic	0	-	-	-	-	
Western viviparous[2]	Opava, Czech Republic	female	normal	49	-	-	-	-	0.0204
			melanic	1	-	-	-	-	
		male	normal	40	50.3 (0.8)	4.0 (0.2)	-	0.080	0.1750
			melanic	7	50.5 (2.8)	3.5 (0.5)	-	0.069	
Western viviparous *Z. v. pannonica*[3]	Bot'any, Slovakia	female	normal	28	-	-	-	-	0.0000
			melanic	0	-	-	-	-	
		male	normal	32	47.6	-	83.2	-	0.0588
			melanic	2	45.3	-	71.0	-	
Western viviparous[4]	Bern, Switzerland	both	normal	262	-	-	-	-	0.0113
		female	melanic	1	55.0	3.6	-	0.065	
		male	melanic	2	46.0 (1.4)	3.0 (1.3)	-	0.065	
Western oviparous *Z. v. louislantzi*[5]	Navarra, Spain	male	normal	54	55.8 (0.3)	3.7 (0.63)	81.7 (1.8)	0.066	0.0005
			melanic	1	53.0	3.4	72.0	0.064	

[1] = this study; [2] = Gvoždík 1999; [3] = Jambrich and Jandzik 2012; [4] = Cavin 1993; [5] = San-Jose, Gonzales-Jimena and Fitze, 2008

in locations including the Czech Republic, Spain, and Slovakia (Gvoždík, 1999; San-Jose, Gonzales-Jimena and Fitze, 2008; Jambrich and Jandzik, 2012). Dorsal colour patterns of adult *Z. vivipara* are mainly composed of shades of brown with stripes, whereas ventral colourations range from pale yellow to dark orange (Vercken et al., 2007). The proportion of melanic individuals has been linked to differences in elevation, vegetation cover, predation, sex, and body condition (Gvoždík, 1999; San-Jose, Gonzales-Jimena and Fitze, 2008; Jambrich and Jandzik, 2012).

In this study we describe the first reported melanism in the oviparous lineage of *Zootoca vivipara carniolica*. We estimate the proportion of melanic individuals within a region in the Austrian alps where *Z. v. vivipara* (Central viviparous II lineage) and *Z. v. carniolica* (Eastern oviparous lineage) are found and compare this frequency, sex bias, and physiological condition we identified to all currently published examples of such information on this rare polymorphism.

Materials and Methods

In total 328 individuals of *Zootoca vivipara* were captured from four sites around the Gailtal valley, Carinthia, Austria. One site was located at the southern face of the Gailtal Alps and three sites at the northern face. Samples were collected between May and August 2016 and 2017. Two sites contained oviparous *Z. vivipara* (site 1: 46.67194ºN, 13.15806ºE, at 1500m of altitude and site 2: 46.60167ºN, 13.14028ºE, at 1400m of altitude) and two sites the viviparous form (site 3: 46.59583ºN, 13.13361ºE, at 1500m of altitude and site 4: 46.58694ºN, 13.20194ºE, at 1400m of altitude). From the 328 individuals captured, 194 were of the oviparous form (99 females, 95 males) and 134 were viviparous (89 females, 45 males). Oviparous and viviparous individuals were identified based on the reproductive traits in females and genome-wide genetic analyses of SNPs in males (Recknagel et al in prep; following approach of Recknagel, Kamenos and Elmer, 2018).

Each individual was photographed dorsally and ventrally, body mass measured using a spring balance, and tail length (TL) and snout-vent length (SVL) measured using digital callipers. Body condition was analysed using the residuals of the linear regression of SVL and body mass, correcting for reproductive mode and sex. Comparison of TL and body condition was restricted to individuals that had fully grown tails, excluding individuals with autotomized tails. No statistical tests were performed, because the sample size for the melanic morph was too small.

Results

In the oviparous lineage, two melanic individuals were found from a total of 194 individuals and both were females (total proportion = 0.0103; proportion just in females = 0.0202). One melanic oviparous female was found from site 1 and one from site 2. While both melanic individuals were very dark, some body colour patterning is still visible (Fig. 1), similar as noted previously at other populations (Jambrick and Jandzik 2012). Throat and ventral colouration varied from light grey to dark grey/bluish. The melanic individual from site 1 was found basking on a dry patch of grass, surrounded by grass patches of up to about 20 cm height. The melanic individual from site 2 was found in a damp section mainly consisting of mosses (*Sphagnum pallustre*) and grasses. No melanic individuals were found from the viviparous lineage (Table 1).

The two melanic individuals measured 46.4 mm and 54.3 mm in SVL. On average, melanic oviparous females were smaller (mean SVL 50.3 mm vs. 58.1 mm) and lighter (mean weight 3.2g vs. 4.8g) than non-melanic oviparous females. Body condition was lower for the melanic females compared to the non-melanic females (0.063 vs. 0.081). However, the average values seen for melanic individuals are within the range of observed values for non-melanic females.

Discussion

As far as we are aware this is the first report of melanic lizards in the eastern oviparous common lizard (*Z. v. carniolica*). Melanism in subpopulations of *Z. vivipara* has been reported in France (Cavin, 1993), Czech Republic (Gvoždík, 1999), Poland (Kolenda et al., 2017), Germany (Petzold, 1978), Montenegro (Iković and Gvozdenović, 2014), Slovakia (Jambrich and Jandzik, 2012), Sweden (Westrin 1985), Spain (San-Jose, Gonzales-Jimena and Fitze, 2008), and now in Austria. This subspecies is sister to all other lineages of *Z. vivipara* (Surget-Groba et al., 2006; Recknagel, Kamenos and Elmer 2018). Since melanism has been reported in numerous more derived and geographically distinct populations across *Z. vivipara*, the presence of melanism in this group suggests that it is an ancestral polymorphism within this species (Table 1). While we did not find any melanic individuals in the Central viviparous II lineage, it is possible that melanic individuals exist in this lineage and will be detected with more sampling effort. However, the fact that the environmental conditions are similar for habitat and altitude for all sites in our study makes it notable that only oviparous melanic individuals were found.

Melanic individuals of ectothermic organisms have been proposed to hold a thermoregulatory advantage over their normal-coloured counterparts, in some cases leading to an increase in body size and overall fitness (Clusella-Trullas, van Wyk and Spotila, 2007; Clusella-Trullas et. al, 2008). Indeed, a study of the melanic morph of the garter snake, *Thamnophis sirtalis*, suggested that larger ectotherms may benefit from this phenomenon (Bittner, King and Kerfin, 2002). Thermal advantage would lead to an increase in body condition, as has been reported in the case of the asp viper, *Vipera aspis* (Castella et al., 2013). Female *Z. v. carniolica* are generally larger than males, and since no melanic males have yet been found in this population, this may support the hypothesis that larger individuals – i.e. females in this case – benefit more from being melanic. We found that body condition was not dramatically different between melanic and non-melanic females. This may indicate that no relationship between melanism and body condition exists in *Zootoca vivipara*, as suggested previously (Gvoždík, 1999; San-Jose, Gonzales-Jimena and Fitze, 2008; Jambrich and Jandzik, 2012), but requires further investigation with larger sample sizes. Similarly, we might have predicted more melanism in viviparous females, as gravid females bask more than non-gravid females (Strugariu and Zamfirescu, 2011; Shine 1980) and thus melanic females, which can heat up more rapidly and have longer pregnancy, would hold an advantage. However we did not find support for that, and in fact the opposite situation: all melanics were oviparous.

Apart from thermoregulation, melanism can affect other traits under selection. Melanin-based colouration is highly pleiotropic and can influence several other phenotypic traits, such as immune and stress response in vertebrates (Mackintosh, 2001; Slominski et al., 2004; Ducrest, Keller and Roulin, 2008). Differences in behavioural traits have also been attributed to melanism, including that darker individuals are often more sexually active, show higher levels of aggression, and are more resistant to stressful environments, however, this also comes with additional costs, such as a higher metabolic rate (Ducrest, Keller and Roulin, 2008).

On the other hand melanism makes individuals more conspicuous, leading to increased predation rates, which constitutes a trade-off to the thermoregulatory advantage and could account for its rarity. This also may be sex-biased. Males have been suggested to be more vulnerable to predation (Forsman, 1995), which could explain the absence of melanic males in this *Z. vivipara* oviparous population. Indeed, black morphs of *Vipera berus* have higher predation rates than lighter morphs (Andrén and Nilson, 1981). However other studies suggest the opposite: a male bias with regards to melanism (Jambrich and Jandzik, 2012), which may be because females bask for longer when they

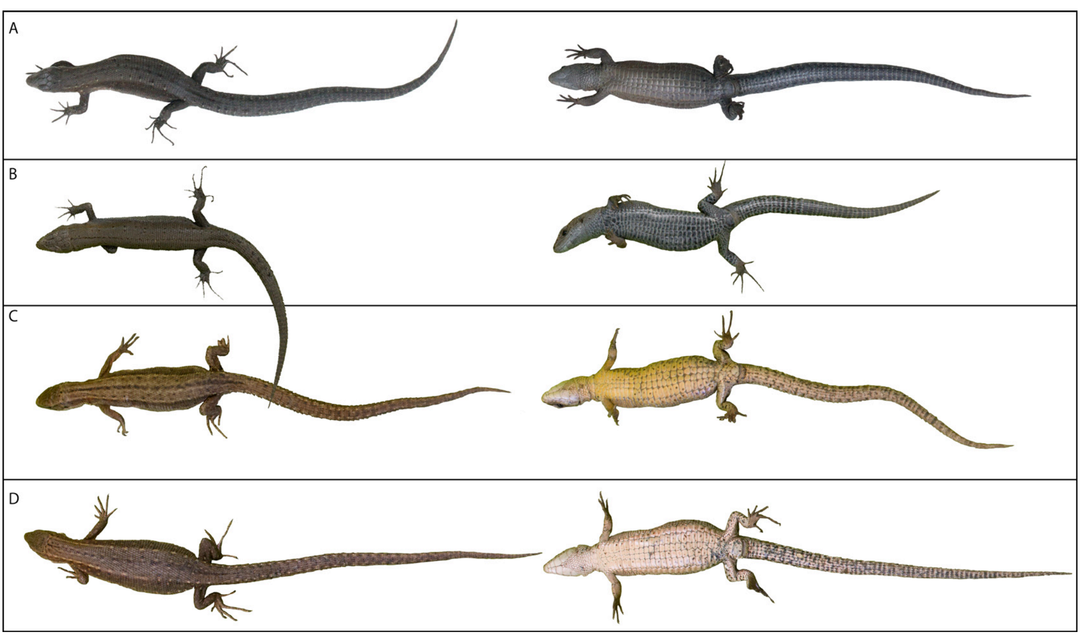

Figure 1. Dorsal and ventral views of melanic (two top individuals A and B) and 'normal' (i.e. non-melanic) coloured (two bottom individuals C and D) female oviparous common lizards (*Zootoca vivipara carniolica*). Ventral colouration of 'normal' oviparous females ranges from white to light yellow, with a variable number of black dots, while melanic individuals are darker, ranging from a pale grey to blueish dark grey. Dorsal colouration of 'normal' individuals ranges from light brown to dark brown, with a visible black line (linear or reticulated) running along the back. The dorsal pattern is still visible in the melanic individual, but less prominent as the background colouration is much darker.

are pregnant and thus rely more on crypsis (Gvoždík, 1999). Excluding the study by Gvoždík (1999) that found several more melanic females (N = 7) compared to males (N = 1), pooling all female (N = 8) and all male (N = 7) individuals reported by other studies results in no clear sex-bias (Petzold, 1978; Cavin, 1993; San-Jose, Gonzales-Jimena and Fitze, 2008; Jambrich and Jandzik, 2012; Iković and Gvozdenović, 2014; Kolenda et al., 2017). Thus far, the evidence is inconclusive and more data is needed to address the issue of a sex bias more rigorously and assess which of the drivers mentioned above favour or disfavour melanism more strongly.

The frequency of melanic common lizards is generally very low across the whole distribution, and any robust interpretation currently suffers from these low sampling sizes. In addition to low sample sizes, most melanic individuals are reported from geographically far apart locations. Across these distances, environments differ drastically, also potentially confounding any general conclusions that might be drawn across common lizards. For future studies, we suggest that sampling efforts should be intensified on populations with known higher frequencies of melanic individuals.

To our knowledge, this study provides evidence of the first instance of melanism reported in the eastern oviparous common lizard subspecies *Z. v. carniolica*. As in other lineages, melanism was very rare. Therefore it is difficult to make conclusions about its association with thermoregulation, sex, body condition, and reproductive mode. However, its presence in this lineage sister to all other lineages may indicate that this is an ancestral polymorphism in *Z. vivipara*. More generally, these findings add to the wider literature on the evolution of melanism among reptile species. Further studies are recommended to increase the number of populations sampled and thus ascertain if there is indeed a relationship between altitude, sex, and melanism.

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