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Frequency and Phenotypic Differences of Melanistic and Normally Colored Common Lizards, *Lacerta* (*Zootoca*) *vivipara* of the Southern Pyrenees (Spain)

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Since the nineteenth century, melanism has attracted the attention of evolutionary biologists, who have focused their research on its evolutionary significance (Cook et al. 2005). Recent studies on mammals and birds corroborate the hypothesis that melanism has a genetic basis and that it is a powerful driver of species evolution (Eizirik et al. 2003; Mundy 2005). Nachman et al. (2003) provided a good example of the adaptive importance of melanism in Rock Pocket Mice, *Chaetodipus intermedius*, which, depending on the background, exhibits different colorations. In order to improve crypsis, light-colored mice live on light-colored rocks while melanistic mice live on dark lava rocks. Nachman et al. (2003) also demonstrated that melanism evolved independently in several mice populations and they found that different mutations located on different genes were responsible for melanism.

As in mammals and birds, melanism is quite common in reptiles (Jandzik 2004; Korsos and Nagy 2006; Mateo and López-Jurado 1994; Pérez-Mellado 1984; Trocsanyi and Korsos 2004; Zuffi 1986). Partial melanism is frequent and partially melanistic individuals typically have ventral scales, which are not completely black coloured (Trocsanyi and Korsos 2004). In some cases the

populations of certain lizards and snakes are formed by a mixture of melanistic and normally coloured animals (Bittner and King 2003), while in other cases entire populations consist only of melanistic or only of normally colored animals (Daniels et al. 2004; Pearse and Pogson 2000). Melanism in Lacerta (Zootoca) vivipara has been described in central and northern Europe, but not in the Pyrenees nor in Spain (Cavin 1993; Kuranova 1989; Malkmus 1976; Petzold 1978; Westrin 1985, 1986). In comparison to other species (Thiesmeier 2002), melanistic common lizards occur at very low frequencies, indicating that melanism in the common lizard might be a rare rather than a well-established polymorphism. An explanation for its rarity might be high selective pressures acting against melanistic individuals (Andrén and Nilson 1981; Husak et al. 2006). Contrary to the rock pocket mice, melanistic and normally coloured common lizards are usually observed in the same habitat and on the same background (Gvozdik 1999). Given that melanistic individuals lose the cryptic dorsal patterns, their dorsal coloration contrasts more with the background (Forsman 1995), making them more visible for predators (Husak et al. 2006). This suggests that melanistic individuals may suffer from increased predation risk (Andrén and Nilson 1981), potentially explaining their low frequency. Additionally, high predation risk may also result in decreased foraging efficiency and in increased physiological stress both negatively affecting body condition (Pérez-Tris et al. 2004; Rands and Cuthill 2001).

On the other hand, it is suggested that the potentially negative effects of melanism on viability and reproduction may be compensated, for example, through increased thermoregulatory capabilities (Gibson and Falls 1979; Rosenblum et al. 2004). This hypothesis is known as the 'thermal melanism hypothesis' (Ellers and Boggs 2002; Parkash and Munjal 1999; Vences 2002). It has been shown to play an important role in many species, including eight lizard species (Daniels et al. 2004). The thermal melanism hypothesis predicts that melanistic individuals should have an advantage under conditions of low temperature since they heat up faster than light-coloured individuals (Clusella Trullas et al. 2007). This indicates that the advantage of melanism should increase with decreasing temperature and thus, that the frequency of melanism should increase with altitude (Clusella Trullas et al. 2007).

To test these hypotheses we used data from several Common



FIG. 1. Photograph of the melanistic *Lacerta vivipara* found in the Southern Pyrenees, Spain.

TABLE 1. Traits of the melanistic male and 54 adult males of the same population of <i>Lacerta vivipara</i> . Given are the mean $\pm$ SE, as well as the
median of the 54 males and the measurements of the melanistic male. We further show to which percentile of the distribution the measurement of the
melanistic male corresponds and we give Wilcoxon signed-ranks tests to test for differences between the melanistic male and the other 54 males.

characters	mean ± SE (range)	median	melanistic male	percentiles	Z	Р
body weight (g)	3.7049 ± 0.6304 (4.8390–2.7140)	3.739	3.3890	20	-0.66144	0.5083
body condition	$-0.0026 \pm 0.0396$ (-0.7345–0.6967)	-0.0064	0.1402	70	0.72443	0.4688
SVL (mm)	55.8333 ± 0.3 (61–52)	56	53	10	-1.30307	0.1926
total length (mm)	137.5741 ± 1.82 (162–113)	138.5	125	20	-0.94568	0.3443
tail length (mm)	81.7407 ± 1.7609 (102–57)	83	72	20-40	-0.75649	0.4494
head length (mm)	10.3611 ± 0.0753 (11.5–9)	10.5	10.5	50-70	0.29572	0.7674
head width (mm)	6.9639 ± 0.0417 (7.6–6.2)	6.95	6.8	30	-0.98198	0.3261
number dorsal scale rows	32.6852 ± 0.3115 (38–27)	32.5	32	40	-0.12781	0.8983
number transverse ventral scale rows	$20.7963 \pm 0.1358(23-19)$	21	21	50-70	0.19958	0.8418
number supralabial scales	6.3889 ± 0.0811 (8–5)	6	6	10-60	-0.66406	0.5066
number infralabial scales	5.4815 ± 0.0825 (6-4)	6	4	0-10	-1.77265	0.0763
number chin shields	4.9259 ± 0.0446 (6-4)	5	5	10-90	0.1264	0.8994
number gular scales	18.2222 ± 0.1786 (22–16)	18	19	70-80	0.77874	0.4361
number collar scales	8.5185 ± 0.1513 (11-6)	9	6	0-10	-1.65811	0.0973
number subdigital lamellae	17.9074 ± 0.157 (21–16)	18	17	10-30	-0.8488	0.396
number femoral pores	10.9815 ± 0.1226 (13–10)	11	11	40–60	0.06652	0.947

Lizard populations to estimate the frequency of melanistic lizards and we compared body condition and several morphological traits between melanistic and typically colored adult male Common Lizards of the same population.

On 19 April 2007, we found a completely melanistic male Common Lizard, Lacerta (Zootoca) vivipara, at the Puerto de Ibañeta, Navarra (Southern Pyrenees, Spain, 43.02158889°N, 1.320416667°W, elev. 1062 m). Although melanism has already been described in Europe for L. vivipara, this is the first time a melanistic individual has been described in the Pyrenees and also the first record for Spain. Thus it is the first record of a melanistic Common Lizard belonging to the oviparous clade, which mainly inhabits the Pyrenees and the Cantabrian mountains (Surget-Groba et al. 2001) and which may belong to a new species that hardly interbreeds with the Common Lizard (Heulin et al 1989; Heulin et al. 1999). The melanistic adult male showed a uniformly black coloration (dorsally and ventrally), with a slightly blacker vertebral line (Fig. 1). The melanistic male was captured in a pre-alpine meadow whose predominant vegetation consists of Ulex gallii and Erica vagans. We discovered him in a small bush (U. gallii), in which we saw and captured several other conspecific lizards. To compare the melanistic individual with typically colored individuals of the same population, we captured an additional 54 adult males. Subsequent to the capture, all lizards were measured following Pérez-Mellado and Gosá (1988). For the analyses of body condition we used the residuals of the regression with SVL as the independent variable and body mass as the dependent variable (Wilson 1991). In order to compare the measurements of the melanistic individual with the other males of the same population, we applied Wilcoxon signed-ranks tests.

To estimate the frequency of melanistic individuals we analyzed data from the population where the melanistic lizard was captured and from six other populations from the Spanish Pyrenees (Provincia de Aragón) that we sampled during spring and summer 2007. The six populations were located at an average elevation of  $1453 \pm 94$  m (range: 1140-1700 m). We captured a total of 515 *L. vivipara* and only one of these individuals was melanistic. Taking into account that not all observed lizards could be captured (captured to not-captured ratio is at most 1:3), we estimate that the frequency of melanistic individuals is maximally 1 per 2000 individuals. This estimate corresponds to a very conservative measurement because melanistic individuals are easier to detect, given that they stand out more against the surrounding vegetation (Andrén and Nilson 1981), and because in population censuses conducted in 2005 and 2006 (ranging from Vielha, Llerida, to Guipuzcoa) no melanistic lizards were observed.

The melanistic male did not significantly differ from the other males in body size, tail length, body condition, or in the number of scales (Table 1). Only the number of infralabial and collar scales was in the lowest percentile (0-10% percentile). Our results on body weight and snout–vent length agree with those obtained by Gvozdik (1999), who did not find differences between melanistic and typically colored common lizards.

The frequency estimation clearly shows that melanistic *L. vivipara* are rare in the Spanish Pyrenees. A potential explanation for the rarity might be provided by the thermal melanism hypothesis (Ellers and Boggs 2002; Parkash and Munjal 1999; Vences 2002). This hypothesis predicts that melanism should provide an advantage in cold climates. For example, in the Western Cape (South Africa) melanistic populations of cordylid lizards are associated with zones of upwelling cold water in the coast, or with mountainous zones with high incidence of fog and cloud cover (Mouton and Oelofsen 1988; Mouton et al. 2002). This hypothesis would also predict that melanism should be more frequent in mountainous habitats. However, our observations do not support this given that the observed melanistic individual was found in the Puerto de

Ibañeta population, which is located at the lowest elevation (1062) m), and no melanistic lizards were found in populations located at higher altitudes and thus in colder habitats. Further, capture effort was higher in the six other populations, which indicates that the frequency of melanistic individuals is likely much lower than in the Puerto de Ibañeta population. Consequently, it is unlikely that thermal advantages explain the observed frequencies. Furthermore, heating rates did not differ between melanistic and normally colored L. vivipara (Gvozdik 1999). It is also unlikely that melanism may have other advantages in the oviparous clade of the L. vivipara: i) melanism was rare; ii) melanism is genetically determined (King 2003; Majerus and Mundy 2003; Mundy 2007; Rosenblum et al. 2004); iii) slight selective advantages should rapidly translate into increased frequencies (Falconer 1960), in short-lived species such as L. vivipara. Alternately, the low frequency might be the result of strong selective pressures against melanistic individuals (Andrén and Nilson 1981). Given that the melanistic male was observed in the same habitat and on the same background as typically colored individuals, he should also be more conspicuous than normally colored individuals (Andrén and Nilson 1981; Forsman 1995). In comparison to typically colored individuals, detection of the melanistic male was markedly easier. However, we could not find evidence that higher conspicuousness might have resulted in decreased body condition (Forsman 1995; Perez-Tris et al. 2004). Further, most of the measured traits were in the 10-90% percentile and only two of fourteen morphological measurements were in the 0-10% percentile. This suggests that the observed melanistic male did not differ from typically colored males in traits other than coloration. Melanism usually results from mutations (Majerus and Mundy 2003; Mundy 2007; Rosenblum et al. 2004) and it has been suggested that in some species melanism is controlled by neutral alleles (Nahrung and Allen 2005), which are maintained at a constant low level. Given that we found no evidence for positive or negative selection, our results are in line with the neutral allele hypothesis, which states that black alleles are not eliminated by natural or sexual selection because black alleles are neither favorable nor unfavorable to survival or reproduction (Nahrung and Allen 2005). This hypothesis might also explain why the frequency of melanistic animals is very low in the Spanish Pyrenees. However, to understand the reasons why melanistic Common Lizards might be very rare, further sampling needs to be carried out to increase the sample size and thus the analytical power.

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## Diet Composition of Bullfrogs, *Rana catesbeiana* (Anura: Ranidae) Introduced into the Venezuelan Andes

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Large size, generalist eating habits, and high reproductive capacity have made the Bullfrog (Rana catesbeiana) an extremely successful invader and important competitor with native species of amphibians (Hecnar and M'Closkey 1997). Increased specialization, competitive inferiority, and predation by Bullfrogs make native species susceptible to declines (Mayuri et al. 2004). Introduction of Bullfrogs has contributed to the decline of native species of Rana in western North America (Bury and Luckenbach 1976; Clarkson and DeVos 1986; Hammerson 1982; Moyle 1973). Rana catesbeiana, is a recently introduced species to the Andean region of Mérida, Venezuela (Barrio 2001) although anecdotal evidence suggests this species has has been present since the mid-1980s. Further, in this introduced population, the pathogen Batrachochytrium dendrobatidis, the agent for chytridomycosis, has been identified in 96% of healthy individuals (Hanselman et al. 2004). This infectious fungal disease is responsible for mass mortality in amphibian species worldwide (Ron and Merino 2000).

Because introduced Bullfrogs have proven to negatively impact native frog populations in other regions, the discovery of Bullfrogs in the Andes of Venezuela prompted us to undertake a variety of studies on their ecology to determine the extent of their impacts on native frog populations. Here, we report the diet of Bullfrogs from a recently established population.

This study was conducted at a 1 km<sup>2</sup> pond in a location known as "La Carbonera" (8.6148333°N, 71.3630556°W, 2360 m elev.), 30 km NW of Mérida city, located in the "Cordillera de Mérida" in the Andes of Venezuela. The surrounding habitat consists mostly of secondary forest (Díaz de Pascual and Chacón-Ortíz 2001).

The Bullfrog was the most common frog in the pond, and was found extensively throughout and around the pond. Fieldwork was conducted monthly from March to November 2002. Large Bullfrogs were captured by air-rifle and small ones by hand and net. Immediately after capture, we measured snout–vent length (SVL; to the nearest 0.1 mm) and mass (to the nearest 0.1 g). Frogs were dissected, the stomachs were removed and the contents placed in 10% formalin for later analysis. Bullfrogs that contained at least one prey item in their stomachs (N = 338) were divided into four categories based on size and morphological characters: 1) Recently-metamorphosed: legs fully developed and tail partially absorbed; 2) Juveniles: tail completely absorbed, 51–69 mm SVL 3) Sub-adults: 70–103 mm SVL; and 4) Adults: 104–180